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MARSHALL'S
PHYSIOLOGY OF REPRODUCTION

MARSHALL'S PHYSIOLOGY OF REPRODUCTION

EDITED BY

A. S. PARKES, C.B.E., M.A., D.SC., SC.D., F.R.S.

National Institute for Medical Research, London

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EDITOR'S PREFACE

TO THE THIRD EDITION

THE appearance of *The Physiology of Reproduction* in 1910 was an event in the history of biological literature. Here, for the first time, was a book containing virtually everything known about the physiological processes involved in reproduction, a book which mapped the present and pointed the way to the future. The work was immediately acclaimed as a masterpiece and placed Marshall in the front rank of British biologists. After the first war a new edition was called for, and appeared in 1922. This edition missed the very rapid expansion of the subject, especially on the endocrinological side, which took place during the 1920's, and by 1935 it was clear that a third edition should be prepared as soon as possible. Marshall realised, however, that the increase of knowledge had placed beyond the powers of a single writer a book of the scope achieved in 1910 and maintained in 1922, and he felt that a number of British workers must be asked to contribute. Arrangements were therefore made for the book to be revised by some fourteen different contributors under my editorship, and to appear in two volumes. By 1939 the preparation of the manuscript was far advanced, but on the outbreak of war it was decided to postpone further work. This decision, for which I was largely responsible, was perhaps a wrong one, but the delays and frustrations of post-war publication could hardly have been foreseen. In 1945 the project was taken up again and the task of revising existing manuscripts and preparing new material was begun. Several new contributors were called upon and estimates of the ultimate size of the book had again to be increased.

The scope of the work is not fundamentally different from that of the 1922 edition, but several new chapters have been required (mainly in Volume I) to accommodate certain aspects of the subject which have grown almost beyond recognition since the book first appeared. Similarly, the general sequence of the book, based so far as possible on the order of physiological events, has been retained, and it has been possible to make the division between the two volumes at what is functionally a convenient point. Volume I, generally speaking, deals with events up to and including fertilisation; Volume II deals with embryonic and foetal development and physiology, lactation, and certain general problems. Unfortunately, it has been necessary to omit much of the older material. In relation to the present literature, the scope of the first edition was enormous and now defies the bibliographic completeness which was Marshall's original aim. In these circumstances, contributors were asked for a workmanlike exposition of the main relevant facts and principles. Moreover, some parts of the subject which once appeared distinct and fell naturally into separate chapters have now tended to run together, and problems of co-ordination between contributors have arisen. A liberal line has been taken in this matter. A particular group of facts may be an integral part of the build-up of more than one chapter, and it would have been impossible, even had it been desirable, to insist upon exclusiveness. It is

to be hoped that the greater value of individual chapters due to their greater completeness will more than offset the disadvantages of some overlap. The reader must judge for himself to what extent these policies have been successful and whether the third edition is a worthy successor to its predecessors.

In existing conditions it seemed likely that there would be difficulty in getting both volumes ready at the same time, and it was decided to treat the two volumes as separate publications, prefaced, pagged, indexed, published, and sold separately. In the end, Volume II was completed first and got so far ahead in the press that its publication before Volume I became inevitable. This sequence, though perhaps not unknown in scientific works, is to be regretted, and it is hoped that the value of the book will not thereby be impaired. In other ways, too, the logistics of this edition have not been easy. It is often said that the difficulty of getting anything done increases as the square of the number of people involved ; there are 18 contributors to the book, as well as the editor, printers and publishers. My apologies are due to Longmans, Green & Co. for the erratic way in which manuscript has come to hand, and to those contributors who, having written their chapters promptly, have been called upon to revise and revise again because of the delays of others. I realise, however, that contributors' delays have been occasioned mainly by striving for perfection, and I am sure that Marshall would have wished to express his appreciative thanks, as I do mine, to all the contributors, who have worked on the book with a care and enthusiasm which it is not always easy to bring to a co-operative work.

A book of this size, published under present conditions, cannot be completely up to date. The material for Volume II was received over a period of three years, and publication will not take place for more than three years after the last manuscript was sent in. In these circumstances, unless page proofs are to be regarded merely as a working draft, very recent material cannot be included. This fact, however, should be judged in proper perspective. The work as a whole embraces the accumulated knowledge of some 2,000 years ; the present edition bridges a gap of nearly 30 years since the previous edition, and will, we hope, be the standard work for a decade or more. Moreover, the value and relevance of biological observations are rarely certain immediately, and a book of this kind should, in our opinion, deal with established and orientated matter. As Marshall himself said, the book cannot be up to date, but it should never be out of date. In these circumstances, no attempt has been made to include stop press news, and the reader who wishes to explore the shifting sands of contemporary effort should consult current reviews rather than this book.

It is sad to record that several contributors or prospective contributors died during the course of preparation of the book. Dr. Batty Griffiths was killed in the North African Campaign in 1943, after having accumulated much material for the chapter on embryology. Sir Joseph Barcroft died in 1947, shortly after completing Chapter 17, and Professor W. H. Newton died in 1949 before receiving page proofs of Chapter 18. Dr. Marshall himself died in February 1949, after having taken a most active and urgent interest in the preparation of the new edition, which occupied a major place in his thoughts during the last few years of his life. It was a tragedy that he did not live to see its publication and that the third edition must be his monument. At the time of Marshall's death there was in press a biographical note for inclusion in Volume 6 of the *Journal of*

EDITOR'S PREFACE

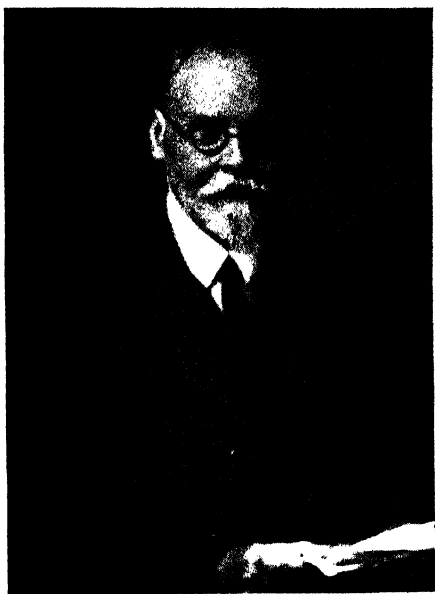
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Endocrinology which was to be dedicated in honour of his 70th birthday. This biography unhappily became an obituary notice, and is reprinted, together with a photograph and a bibliography of his writings, as part of the introduction to this edition.

September 1950.

A. S. PARKES,
National Institute for Medical Research,
London, N.W. 7.

F. H. A. MARSHALL, C.B.E., M.A., Sc.D., F.R.S.



(J. Russell & Sons)

H. A. MARSHALL

It was intended to recognise the 70th birthday of one of our most distinguished biologists, Dr. F. H. A. Marshall, by dedicating Volume 6 of the *Journal of Endocrinology* in his honour. Unfortunately, this plan to mark a happy occasion was forestalled by his death, which occurred suddenly on 5 February, 1949.

Recent developments, by their nearness, have tended to overshadow the pioneer work in which Marshall played so important a part, and it is with appreciation that the writer recalls, for a new generation of biologists, the achievements of his friend and one-time tutor.

Marshall's name is associated, mainly or even exclusively, with the study of the physiology of reproduction in its many branches. He was preceded in this field by another pioneer, Walter Heape, who was completing his classic studies of the primate uterus and collecting material for

his analysis of the oestrous cycle at the time when Marshall was an undergraduate in Cambridge. The two men did not meet until later, but they corresponded freely, and Heape's morphological studies and extensive knowledge of living animals were of great value to Marshall in his early days. Heape, however, was not a physiologist—he seems, for instance, to have regarded the ovary merely as a producer of eggs—and it is to Marshall that we owe the foundations of our present knowledge of the role of internal secretions in the reproductive processes.

Marshall's debut was characteristic of an age in which research was a calling rather than a profession; a precarious calling to which young men were drawn by interest, not by ambition. In 1900, the late Lord Carmichael gave £200 to Prof. Cossar Ewart, Professor of Natural History in the University of Edinburgh, to enable a Research Student to assist with his long-term experiments on telegony, and to undertake work on reproduction in farm animals. Cossar Ewart's inquiries resulted in Marshall accepting an invitation to go to Edinburgh. The work on telegony, which involved a detailed study of the hair of the Equidae, was uninteresting and unproductive, and Marshall turned his attention to research on the sexual cycle in the sheep, for which he was to make use of Ewart's farm at Penicuik. This work was the subject of his first important scientific publication, which appeared in the *Transactions of the Royal Society* in 1903. Similar studies followed with Schäfer on the oestrous cycle in the ferret (1904), and with Jolly on the oestrous

cycle in the dog (1905). A communication on the ovary as an organ of internal secretion, written in collaboration with Jolly and published in the *Transactions of the Royal Society* in 1905, constituted the first serious attempt to correlate the changes in the uterus during the reproductive cycle with the cyclic production of different internal secretions by the ovary.

Marshall returned to Cambridge in 1908, and continued his work on the physiology of reproduction. During the war, 1914–18, he studied, on behalf of the Ministries of Food and Agriculture, such problems as the most economic age and condition at which to slaughter cattle. Subsequently, Marshall's researches have dealt mainly with the influence of exteroceptive factors—light, climate, etc.—on the reproductive processes, and in 1936 he made this the subject of his Croonian Lecture. He was also intensely interested in the courtship and breeding behaviour of birds, on which he was a considerable authority.

Marshall spent a great deal of time preparing the first edition of *The Physiology of Reproduction* in which he gathered together all relevant knowledge concerning the breeding season, cyclic changes in the reproductive organs, fertilisation, fertility, lactation, and so on. The book was published by Longmans, Green & Co. in 1910, and immediately achieved success. A second edition was called for after the 1914–18 war. This second edition, published in 1922, was necessarily much larger than the first, for since 1910 knowledge of the reproductive processes in mammals and lower vertebrates had increased considerably. A third edition, largely rewritten by Marshall's pupils and colleagues, and again much enlarged, is now in the course of publication.

Marshall's main contribution to biology has been twofold. In the first place, the stimulating synthesis of existing knowledge embodied in the first edition of *The Physiology of Reproduction* gave impetus and balance to a branch of biology sadly lacking in both. The fact that between the wars the physiology of reproduction became a subject of intensive study by scientists in all countries was due in no small measure to the stimulus given by Marshall's writings. In the second place, Marshall's remarkable prescience in deducing correctly the existence and respective roles of follicular and luteal hormones long before either was isolated, or even obtained in active extracts, had a profound influence on the development of the subject. During the early years of the century Ludwig Fraenkel in Breslau and P. Bouin in Strasbourg, working on the rabbit, concentrated attention on the corpus luteum; in the 1920's intensive study of the follicular hormone by Edgar Allen and his colleagues in the U.S.A. swung attention to the follicular apparatus. It fell to the English school of workers, under Marshall's influence, to hold the balance until the final demonstration and isolation of the corpus luteum hormone in G. W. Corner's Rochester laboratory completed the working out of his early predictions.

Scientists are of many kinds, but inspiration flows most fruitfully from those who are able, by some gift withheld from lesser men, to divine the richness of uncharted country and sense the vital landmarks. Thus do they avoid the barren places and the morasses of unimportant detail which engulf so many. To these, discovery is an art rather than a science, a matter of instinct rather than of intellectual machinery. Such was Marshall.

A. S. P.

20 October, 1948.

Revised 10 February, 1949.

CURRICULUM VITAE

FRANCIS HUGH ADAM MARSHALL was the younger son of the late Thomas Marshall, J.P., and was born at High Wycombe on 11 July, 1878. He was educated at St. Mark's School, Windsor, and privately. After a short time at University College, London, he went to Christ's College, Cambridge, in 1896, and took the Natural Science Tripos in 1899. During the latter part of the time in Edinburgh Marshall became Carnegie Fellow and Lecturer on the physiology of reproduction. After his return to Cambridge he was Lecturer and then Reader in Physiology, in the School of Agriculture, for more than 35 years, until 1943. For a short period, Marshall followed the late Prof. T. B. Wood in directing the Institute of Animal Nutrition. He became in succession, Fellow, Tutor, Dean, and finally, in 1940, Vice-Master of Christ's College. He was a Proctor in 1911-12 and afterwards served on the Council of the Senate. Marshall was an original member of the Council of Management of the *Journal of Endocrinology* and was one of the four honorary members of the Society for Endocrinology. For many years he was an editor of the *Journal of Agricultural Science*, and finally became principal editor. Marshall's scientific distinctions included D.Sc. (Edin.), Sc.D. (Cantab.), D.Sc. (Hon. Manch.), LL.D. (Hon. Edin.), F.R.S. (1920), C.B.E. (1933), Council of the Royal Society (1933-5), Baly Medal of the Royal College of Physicians (1935), Croonian Lecturer (Royal Society) 1936, and Royal Medal (Royal Society) 1940.

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EXCERPTS FROM THE INTRODUCTION TO THE FIRST EDITION

SINCE the time when physiology first became an organised science many volumes have been written on the digestive, excretory, nervous, and other systems of the body, but no attempt has yet been made to supply those interested in the reproductive processes with a comprehensive treatise dealing with this branch of knowledge. Indeed, in most text-books on physiology now commonly in use either the section devoted to the reproductive organs is restricted to a few final pages seldom free from error, or else the subject is entirely omitted. Yet generative physiology forms the basis of gynaecological science, and must ever bear a close relation to the study of animal breeding. In writing the present volume, therefore, I have been actuated by the desire to supply what appears to me to be a real deficiency ; and in doing so I have attempted, however inadequately, to co-ordinate or give a connected account of various groups of ascertained facts which hitherto have not been brought into relation. For this purpose I have had occasion to refer to many books and memoirs dealing with subjects that at first sight might have been supposed to differ considerably. Thus, works on zoology and anatomy, obstetrics and gynaecology, physiology and agriculture, anthropology and statistics, have been consulted for such observations and records as seemed to have a bearing on the problems of reproduction.

The present volume is addressed primarily to the trained biologist, but it is hoped that it may be of interest also to medical men engaged in gynaecological practice, as well as to veterinarians and breeders of animals. As a general rule, I have confined myself to the physiology of generation among the higher forms, and more particularly the Mammalia, but I have not hesitated to discuss the reproductive processes in the Invertebrata on cases where they seemed likely to elucidate the more complex phenomena displayed by the higher animals. The all-important questions of heredity and variation, although intimately connected with the study of reproduction, are not here touched upon, excepting for the merest reference, since these subjects have been dealt with in various recent works, and any attempt to include them would have involved the writing of a far larger book. Similarly, the subject matter of cytology, as treated in such works as Professor Wilson's volume on the cell, is also for the most part excluded.

It may be objected that, for a book on physiology, too much space is devoted to the morphological side of the subject. This has been done purposely, since it seemed impossible to deal adequately with the physiological significance of the various sexual processes without describing the anatomical changes which these processes involve.

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The late Professor W. H. Newton, M.D., D.Sc., *Edinburgh.*

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CHAPTER 14

CLEAVAGE, EARLY DEVELOPMENT AND IMPLANTATION OF THE EGG

By J. D. BOYD and W. J. HAMILTON

I. GERM-CELLS

In the various vertebrates, there are marked differences in the form, the structure, and, to a lesser extent, in the physiological activities of the male and female germ-cells, sperms and ova, just as there are differences between the adult animals. Both male and female germ-cells are highly specialised, and the form and structure of these cells are adapted to, or determined by, the functions they are destined to perform.

Sperms

The sperms usually possess more distinctive features than the ova, and these appear to be principally adaptations for the special problems with which the sperms must cope in order to reach and penetrate the ovum and its membranes. In the sperm the amount of cytoplasm has been reduced to a minimum and each is provided with a flagellum or tail which, by its activity, renders the sperm highly motile. Frequently the sperm head is provided with a special mechanism for perforating the ovum and its membranes. After the fertilising sperm has entered an egg, its structural peculiarities do not appear to be concerned in any way with the further development of the zygote. Its nuclear chromosomes, however, which are transformed into the male pronucleus in the cytoplasm of the ovum, play an equally important part with those of the pronucleus of the ovum (female pronucleus) in determining the genetic constitution of the zygote. (For the structure and physiology of the sperm, *see* Chap. 7.)

Ova

The ova, on the other hand, possess a large amount of cytoplasm in which reserve material is stored in the form of relatively inert yolk, or deutoplasm. In addition, ova usually have protective envelopes called membranes. Since they possess no motile organ, they can only be moved passively. Although in vertebrates ova do not show such marked structural peculiarities as the sperms, there are distinct differences in their size and membranes not only in the different groups (Figs. 14. 1-4) but even within a given group (Fig. 14. 5). The differences in size depend almost entirely on the amount of the deutoplasm.

The primitive method of reproduction in the Metazoa appears to have been that in which a large number of eggs, with relatively little yolk, was produced. Such a method, although a severe drain on the maternal organism, undoubtedly

facilitates wide dispersal of the eggs. As there can be little stored material in such eggs a minute free-swimming larval stage in development is necessary. In the course of vertebrate evolution the general tendency has been to increase egg size and reduce the number of eggs produced and there is a concomitant tendency to prolong the developmental period. These trends reach their climax in the reptiles and birds in which the larval stage has been completely suppressed. In the mammals with their specialised viviparous mechanisms egg size has been secondarily reduced, only slightly in Prototheria, but markedly in Eutheria.

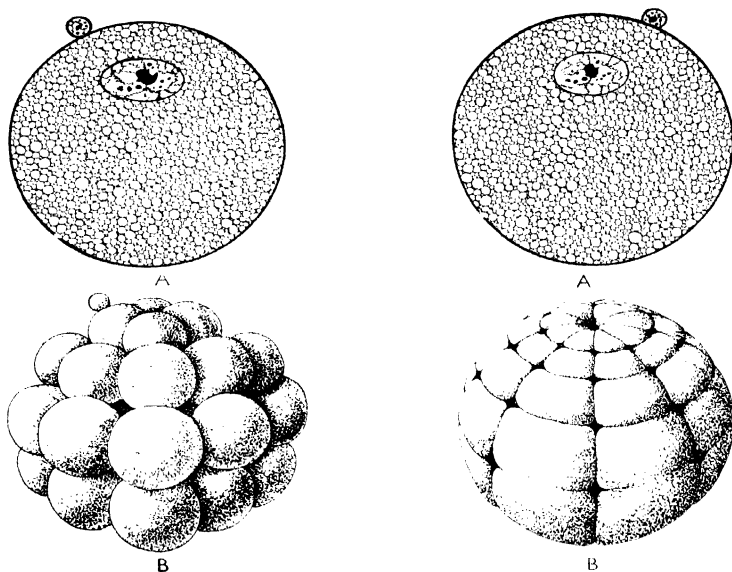


Fig. 14. 1—A. Schematic section of mature egg of *Amphioxus*. B. Early blastula of *Amphioxus*.

Fig. 14. 2 A. Schematic section of mature amphibian egg. B. Early amphibian blastula stage.

The size of the ripe eggs, excluding their membranes, range from diameters of about $100\ \mu$ in *Amphioxus* and mammals (range of $80\ \mu$ to $150\ \mu$) to about $85\ \text{mm}$. or even more in the ostrich and mackerel shark. Such enormous eggs represent the largest single cells in the existing animal kingdom. Even the smallest mature vertebrate egg is relatively large when compared with the somatic cells of the organism from which it is derived. There are also marked differences in the nature, size and distribution of the yolk granules (deutoplasm) of different vertebrate eggs. On the basis of their size and the arrangement of their yolk granules the eggs of vertebrates may be classified into three main groups (Table I). These are (1) *Miolecithal* (e.g. the eggs of *Amphioxus*, Metatheria and Eutheria) with very little yolk; (2) *Medialecithal* (e.g. the eggs of Petromyzontidae, a number of fish groups including the Dipnoi, and most of the Amphibia) with intermediate amounts of yolk; (3) *Megalecithal* (e.g. the yolk-rich eggs of other fishes, apodan Amphibia, reptiles, birds and monotremes) with a large amount of yolk. The amount of yolk in the egg, as will be seen later, has a profound influence on cleavage and the

TABLE I
TYPES OF OVA AND CLEAVAGE

<i>Amount and distribution of yolk.</i>	Little, evenly distributed.	Medium, less near one polar region.	Much, none at one pole.
<i>Common name of egg type.</i>	Yolk-poor.	Medium yolked.	Yolk-rich.
<i>Technical name of egg type and meaning.</i>	Miolecithal (less-yolk).	Medialecithal (medium-yolk).	Megalecithal (large-yolk).
<i>Position of egg nucleus.</i>	Near centre.	Near one pole.	At one pole.
<i>Relative amount and position of active cytoplasm.</i>	Much throughout the ovum.	Medium amount, but near nuclear or animal pole.	Very little and entirely at nuclear or animal pole.
<i>Type of cleavage.</i>	Complete, nearly equal.	Complete, unequal.	Incomplete, unequal.
<i>Technical term for cleavage type.</i>	Holoblastic.	Holoblastic.	Discoid (meroblastic).
<i>Familiar examples.</i>	<i>Amphioxus</i> , Man.	"Ganoid" fishes, frogs and toads.	Reptiles and birds.
<i>Main chordate groups exhibiting each type.</i>	Tunicata, Cephalochordata, Marsupialia, Eutheria (placental mammals).	Petromyzontidae, Polypteridae, Chondrostei, Amiidae, Holocephali, Dipnoi, Amphibia (except Apoda).	Myxinidae, Euselachii, Lepidosteidae, Teleostei, Apoda, Reptilia, Aves, Monotremata.

subsequent developmental history of the resulting embryo. For details of the sizes and weights of infra-mammalian vertebrate eggs, *see* Kerr (1919), Roch (Tunicata, 1930), Franz (Acrania, 1930), Schiemenz (Fish, 1930), Ahl (Amphibia and Reptilia, 1930), Heinroth (Aves, 1930) and Brinley (Fish, 1938). The sizes of mammalian eggs will be considered in detail subsequently, but summaries can be found in Hartman (1929a), Amoroso *et al.* (1942) and Hamilton and Day (1945).

In form the ova of vertebrates are usually spherical, but the surrounding membranes may have quite bizarre shapes (e.g. in the Euselachii), and in the Sauropsida they take the characteristic form of a prolate spheroid. For an analysis of the shape of the avian "egg" the reader is referred to Thompson (1942) and to Needham (1931).

The differences between the eggs of different vertebrates in the amount of deutoplasm and in the various egg membranes may be regarded as adaptations to the different environments in which they must develop. Or, conversely, it may be that they can only develop in particular environments or micro-climates, because of their possession of the requisite stored material and protective envelopes.

Whatever the ultimate explanation, the absence of large amounts of yolk in the eggs of some oviparous types necessitates that, unless there is a diapause, the development period must be short. Consequently the embryos are usually hatched as larvae which lead an active life and derive nutriment from their external environment before assuming, either by sudden metamorphosis or by more gradual growth

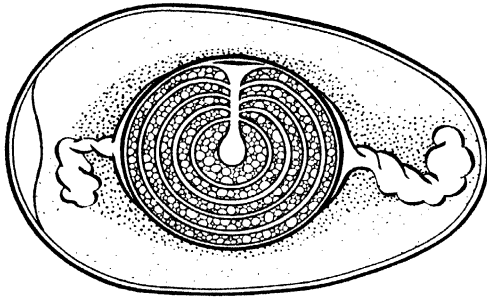


Fig. 14. 3 - Schematic section of avian " "

changes, their definitive adult form. On the other hand, eggs in oviparous types with large amounts of yolk, permit of a longer period of development before hatching. This is particularly true in those types, e.g. birds, where the yolk is supplemented by further nutritive material in the form of albumen (Fig. 14. 3). In the development of such vertebrates adult-like characters appear before hatching. Even within a given group, however, there is considerable variability.

Thus in birds there are *nidicolous* types, with short incubation periods, and *nidifugous* types, with long incubation periods. In the latter the young are well clothed in feathers at hatching and can feed themselves.

When the eggs are not "laid," but are retained for a shorter or longer period inside the maternal body, the latter can supply water and nutritive materials to the developing organism. In some such types, called ovoviviparous (e.g. certain fishes,

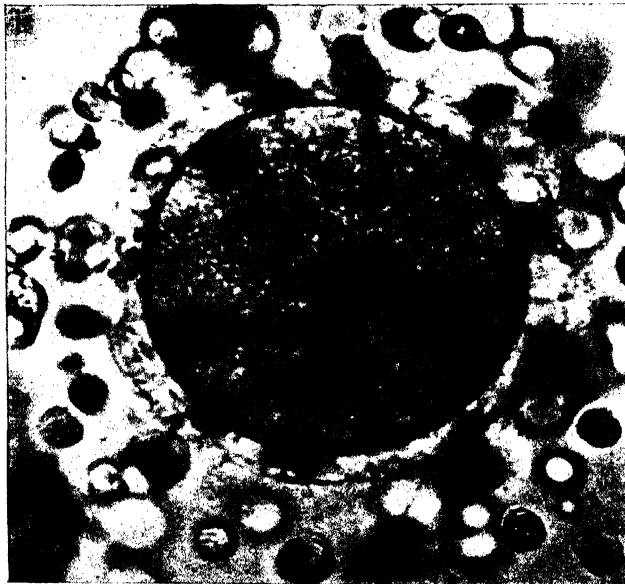


Fig. 14. 4 - Photomicrograph of a living human ovum surrounded by the corona radiata recovered from the uterine tube. $\times 480$. (From Hamilton, Boyd and Mossman, 1945.)

amphibians and reptiles), the retained egg is often very little different from the eggs of related oviparous types and there is very little exchange between maternal and embryonic organisms except water and respiratory gases. In other types, however (e.g. certain other fishes, reptiles, Metatheria and, pre-eminently, Eutheria), the egg is not only retained within the maternal organism, but a special mechanism, the placenta, is developed to permit of the interchange of nutritive material, respiratory gases and the end products of nitrogenous metabolism between the maternal and the developing organisms. This method of development is called viviparity. Eutherian mammals are so markedly viviparous that although they were almost certainly derived from reptilian ancestors with megalecithal eggs, their ova are of the miolecithal type (but *see* de Lange, 1916 and 1923, for an alternative interpretation). Eutherian embryos are dependent, in the earlier stages of development, upon uterine secretion and, in the later stages, on one or other of the various types of placental mechanism for receiving their nutrition from the maternal organism.

The adjective cleidoic has been used by Needham (1942, for summary) to designate those eggs supplied with ample reserves of nutritive material and water, and, therefore, more completely closed off from their environment. Such eggs are not so dependent upon their surroundings as eggs less well endowed with stored substances. The cleidoic eggs of birds, for example, contain within themselves practically all the materials necessary for their development, but they are still dependent upon the environment for oxygen and possibly some intake of water. Non-cleidoic eggs, on the other hand, are not initially supplied with sufficient inorganic material and water to build a complete embryo. Many eggs laid in water by invertebrates and fishes are of this type, and the embryos of such eggs obtain many of their necessary requirements from the aqueous environment. Mammalian eggs must also be classed as non-cleidoic as they are dependent, in the early stages of development, upon organic and inorganic materials absorbed from the secretions of maternal uterine glands and are later dependent upon the placental mechanism for all the substances they require for growth and differentiation.

As Needham has pointed out, the metabolism of the cleidoic eggs of birds and reptiles is markedly different from that of the non-cleidoic eggs of fishes and Amphibia. Thus the Sauropsida metabolise less protein and more fat during their embryonic life than do the Ichthyopsida. Another noteworthy feature of the cleidoic egg is the rapidity of its incubation as compared with the gestation period of the mammal, for it requires longer to produce a mammal of a given birth weight than a bird of an equivalent hatching weight.

II. CYTOPLASM OF THE EGG

During the growth of the oocyte, anabolic processes are in excess of the catabolic phenomena; consequently, by the time that the egg has reached maturity, it has accumulated reserves of material—deutoplasm. This is later utilised to provide energy and substance for development. When compared with the ordinary somatic cell, the ovum is large; this is true even when the ovum is relatively very small, as in *Amphioxus* and the eutherian mammals. In other groups it may be very large indeed, the size being due chiefly to stored deutoplasm. The ovum is incapable of locomotion; during certain stages (e.g. at fertilisation), however, it can undergo a wave of contraction. The nucleus of the ovum is unusually large with a

distinct birefringent nuclear membrane. It is frequently surrounded by a clear area of cytoplasm free from inclusions. When the vitellus of the egg is freed from stored reserves (deutoplasm), the cytoplasm (hyaloplasm) is essentially similar in appearance and in chemical structure to the cytoplasm of somatic cells. The cytoplasm of ova, however, is much greater in amount, though the presence of a large amount of deutoplasm, for which the cytoplasm forms a matrix or ground substance, often masks this fact. The cytoplasmic distribution and character are not completely homogeneous in the egg. The surface layer (cortex, "coat" or superficial pellicle) forms a limiting film with special orientation of the ultra-microscopic micellae. The "coat" plays an important part in later development (*see* Holtfreter, 1943a; Pasteels, 1946) and may be concerned in the establishment of axial symmetry. Beneath the cortex there is usually a highly viscous cytoplasmic sub-cortical zone which is continued into the interior of the egg as its general cytoplasm or hyaloplasm. Monné (1944), using the polarising microscope, recognises two distinct phases in the cytoplasm of the miocithal sea-urchin egg — a fluid *enchylema* in which the yolk granules and the mitochondria are suspended and an *orientated phase*, of more or less fixed structure, which is constituted by elongated fibrillae rich in ribonucleic acid. Monné believes that the birefringency of the cortex is due to the parallel orientation of these fibrillae in this situation. As will be seen later ribonucleic acid proteins appear to be intimately concerned in the synthesis of other proteins and to play an important rôle in future morphogenesis. There is no absolute distinct boundary between cytoplasm and deutoplasm, for a transitional zone is nearly always present. In miocithal eggs they may indeed be intimately mixed.

Wetzel (1907) has indicated that in general four categories of substances may be identified in eggs. These are (1) the protoplasm of the egg-cell, (2) the constituents of the protoplasm of the completed embryo, (3) the constituents of the "paraplasm" and (4) the substances used as a source of metabolic energy during development. The reserves of deutoplasm are of a protein, lipide, carbohydrate, or mixed nature. The protein material forms the vitelline granules, or, in large eggs, the vitelline platelets. These are usually phosphoproteins combined with lipide and are anisotropic. Owing to the fact that these protein reserve substances have a relatively high specific weight, they are driven, in centrifuged eggs, to the centrifugal pole. The stored fats of the egg are in the form of droplets of varying size which can be stained with osmic acid, Sudan III and other fat stains. As they have a low specific gravity they accumulate at the centripetal pole when the egg is centrifuged. In some eggs (e.g. those of fishes) the fat is in the form of discrete oil droplets which may be pigmented. Little is known about the carbohydrate content of most eggs, but, in suitably fixed and stained oocytes, glycogen appears as fine, evenly scattered granules. Carbohydrate undoubtedly is also present in conjugated protein materials.

Owing to the varying size and specific gravity of the particles of deutoplasm there is frequently a vitelline gradient in the mature egg, the size of the granules increasing from the animal to the vegetal pole. In many eggs there are pigmented inclusions of a different and often unknown nature. The pigment may be concentrated at one of the poles (*see* later) or scattered throughout the cytoplasm, or related to the cortical and sub-cortical regions. In some types it appears to be soluble in lipoids and is therefore associated with the oil droplets of the fatty yolk.

For a summary of the extensive literature on the cytoplasmic inclusions, such as Golgi apparatus, mitochondria and centrosomes, see Chap. 5.

III. COMPOSITION OF VERTEBRATE EGGS

Amphioxus

The mature eggs are fairly uniform in size ; in fixed, stained and mounted material, they are about 100-120 microns in diameter (Franz, 1930 ; Conklin, 1932). Before the first maturation division, the eccentrically situated nucleus has a diameter approximately half that of the egg. The cytoplasm is semi-fluid and very labile. It is arranged into a peripheral zone, relatively free of yolk spherules, and a central yolk-laden area. During the maturation divisions, the size of the nucleus is markedly reduced and there is left surrounding it an area of yolk-free hyaloplasm. After fertilisation, the peripheral layer of cytoplasm migrates to the area of sperm entry, and there constitutes the presumptive mesodermal crescent (see p. 21). These changes in the egg of *Amphioxus* are very similar to the corresponding, but better known, phenomena occurring in the eggs of the Tunicata (see Conklin, 1905, 1932, and Daleq, 1938, 1941). In these prochordates the egg and cleavage have been intensively studied by vital staining and experimental techniques.

Pisces

On the basis of their structure and the manner in which they develop, the eggs of fishes are classified into two main types, pelagic and demersal. Pelagic eggs are buoyant and are generally provided with a thin non-adhesive membrane. Demersal eggs, on the other hand, are heavy and sink in water ; they have a hard, smooth or adhesive membrane. They are found in both marine and fresh water species.

Eggs of the pelagic type are much smaller than demersal ones and are usually markedly translucent, which facts help to make them inconspicuous to potential enemies. The pelagic eggs of the plaice (*Pleuronectes*), are amongst the largest of this type, but they never exceed 2 mm. in diameter. Many pelagic eggs possess conspicuous oil globules, frequently a single large one. The presence of characteristic pigment in the globules is of help in identifying such eggs. The function of the oil is doubtful. It appears to give buoyancy to the egg, and may be stored food, though it often remains as a residue after the complete absorption of the yolk. Needham (1931) has suggested that the oil may be a hydrocarbon and not a fat. Demersal eggs are usually large in species that spawn in fresh water. *Gymnarchus* has eggs of up to 10 mm. in diameter, while those of some of the Arridae occasionally exceed 15 mm. Amongst marine fishes the eggs of the wolf-fish (*Anarrhichas*), are 6 mm. in diameter, but most demersal marine eggs are much smaller.

In addition to the pelagic and demersal eggs of oviparous fishes, there are the eggs of the many ovoviviparous and viviparous species. The eggs of such species vary much in the amount of deutoplasm. Two members of the viviparous Poeciliid family of Cyprinodont fishes (*Heterandria formosa* and *Aulophallus elongatus*), possess eggs which appear to be the smallest of any fish. They measure not more than 0.4 mm. in the former and 0.6 mm. in the latter (Scrimshaw, 1944a and b and 1945). This small size has been correlated with the viviparous habits in these species. Scrimshaw (1946) has demonstrated, however, that viviparity is not the

cause of this, since eggs of other members of the same family are much larger. For size of eggs of fishes *see* McIntosh and Masterman (1897), Breder (1929), Schiemenz (1930), Brinley (1938) and Scrimshaw (1946).

The chemical nature of the inclusions of fishes' eggs has not been intensively studied. Summaries of the literature are given by Needham (1931 and 1942). A striking fact is that the yolk material in fishes' eggs is predominantly protein in nature. There are considerable variations in the fat content of the eggs of different fishes, but the deutoplasm is always predominantly protein.

Amphibia

Amphibian eggs are mostly of the medialecithal type, with diameters, usually, of from 2 to 3 mm. Within a single species, however, there is often considerable variation (*see* Ahl, 1930, and Noble, 1931). In most species the eggs are laid in water, but in some they are deposited on moist terrestrial surroundings, and may elide the tadpole larval stage during their development. The eggs of the Apoda are megalecithal and range up to 10 mm. in diameter. Several amphibian species, e.g. *Salamandra* and the caecilian *Typhlonectes*, are ovoviviparous.

As a prototype for the study of vertebrate development, much work has been published on the structure and, to a lesser extent, on the chemistry of the amphibian egg, but there is still much uncertainty on its detailed cytology and its cytochemistry. All amphibian eggs show a distinct polarity with deeper pigmentation, and more cytoplasm at the animal pole and abundance of deutoplasm at the vegetal pole. In many of the eggs the distribution of the superficial pigmentation is such that, even in the unfertilised condition, they are bilaterally symmetrical (Banki, 1927 ; Pasteels, 1938 ; Lehmann, 1945). Banki believes that it is possible to identify different " plasma " regions, such as have been identified in *Amphioxus* and many invertebrates. In particular Lehmann has stressed the existence of a marginal zone of autonomous subcortical plasma, which, he considers, has a special rôle in development, having as its function the formation of the future dorsal lip of the blastopore. Pasteels (1946) has criticised Lehmann's conclusions in this regard. He subdivides the unsegmented, mature Axolotl egg into four cytoplasmic areas : (1) the pigmented cap, (2) the region of the " noyau central," (3) the massive white deutoplasm of the vegetal pole, and (4) the " cheminée de maturation," a narrow radial streak of unpigmented cytoplasm that underlies the animal pole and is related to the nucleus. The *pigmented cap* comprises the whole of the animal part (with the exception of the " cheminée ") and the margin of the egg, corresponding precisely with the limit of cortical pigment. Its lower surface, which rests on the deutoplasm of the central nucleus, is concave. In structure, the cap is almost homogeneous, consisting of small dispersed deutoplasmic granules, abundant microsomes charged with ribonucleic acid and many pigment granules (" *braune Dotter* " of Penners and Schleip, 1928) which give the characteristic greyish coloration to the fresh egg. The " *noyau central* " consists of a comparatively dense aggregation of yolk granules of variable size, rather heterogeneous arrangement and containing small strongly refringent particles. Pigment and ribonucleic microsomes are absent. The vegetal pole deutoplasm has the form of a thick bowl, the concavity of which contains the " central nucleus," while the edges are in contact with the pigmented cap. Its structure is homogeneous, consisting of large uniform yolk granules, amongst which are occa-

sional pigment granules. The "*cheminée de maturation*" possesses essentially the same structure as the pigment cap. It has, however, very little pigment and consists of cytoplasm almost devoid of granules.

Essentially similar descriptions to this of Pasteels for the axolotl have been given by Roux (1903) and by Ancel and Vintemberger (1944) for the frog's egg. Here, however, the "*noyau central*" is pigmented and the lower "horns" of the pigment cap are insinuated more deeply between the marginal cortex and the vegetal deutoplasmic region.

All of the cytoplasmic materials in the amphibian egg can be readily dislodged by gravity or centrifugalisation, which makes it unlikely that there is a fixed architecture in the cytoplasm such as Monné (1944) has indicated for the sea-urchin egg. Experimental work, however, has demonstrated that there is some stable structure, with a dorso-ventral organisation, which is not affected by the alteration in ovular materials due to gravity. It seems that this stable structure must be looked for in the superficial cortex, "coat," or pellicle of the ovum (see Ancel and Vintemberger, 1939; Holtfreter, 1943a and b and 1944, and Pasteels, 1946).

Holtfreter (1946a and b) has recently described the physico-chemical characteristics of the yolk platelets and the lipo-protein bodies of amphibian eggs. He gives a full account of their reactions to various fixatives, stains and solvents and of the effects of changes in the pH and in the electrolyte concentration. His results suggest that disintegration of the yolk platelets and a simultaneous conversion of the lipo-protein bodies ("lipochondria") into fat droplets are normal features of amphibian development.

Reptilia

Reptilian eggs are megalecithal and for the most part reptiles are oviparous, though a number of them are ovoviviparous and some can be regarded as truly viviparous (see Chap 15). The ova possess complex membranes, including a shell of some sort (see p. 17). The extreme diameters of the "eggs" (i.e. ova with membranes) range from 5 × 7 mm. in a lizard (*Leiolopisma laterale*) to 120 × 60 mm. in a snake (*Python molurus*) (Table II). The eggs are often very elongated, e.g. those of *Typhlops brahminus*, in which they measure 12-14 × 3-4 mm. Very little work has been done on the histological structure and chemical composition of reptilian eggs. In the eggs of certain tortoises and lizards, there is twice as much protein as fat, while in those of certain snakes the amount is three times as great.

Aves

Avian eggs are always of the megalecithal type. The vitellus (yolk) or "ovum" varies in size from an estimated diameter of 6 mm. in a humming-bird to 80 mm. in the ostrich. Measurements for the whole egg in the former, are 14 mm. × 9.5 mm. and in the latter 155 mm. × 130 mm. The vitellus of the egg of the now extinct *Aepyornis* is estimated to have measured 175 mm.; its shell measures 37.5 cm. × 24 cm. and has a cubic content of approximately 2 gallons.

The vitellus of the hen's egg averages 40 mm. in diameter and weighs from 16 to 17 gms. The whole egg weighs from 55 to 60 gms. The eggs, of course, vary in size and weight according to the breed and the age of the hen and also to the time of year.

TABLE II
COMPARATIVE DIMENSIONS OF SOME VERTEBRATE EGGS

Animal	Diameter of Vitellus (in mm)	Volume of Vitellus (in cubic mm)	Authority
AMPHIOXUS	0.1012	0.000523— 0.000904	Franz, 1930 Conklin, 1932
PISCES			
SMALLEST Cyprinodonts (<i>Heterandria formosa</i>)	0.4	0.0335	Schrimshaw, 1944a & b and 1945
LARGEST <i>Chlamydosclache</i>	150 (approx.)	1,766,000	Wilson, 1925
AMPHIBIA			
SMALLEST			
<i>Bufo calamita</i> <i>Rana arvalis</i>	0.7	0.15	(Bedriaga, 1891 Hempelmann, 1905)
LARGEST <i>Crotaphyla bubalis</i> <i>Nototriton orificum</i> and <i>fissipes</i>	10	524	Werner 1922 Nieden 1923
REPTILIA			
SMALLEST <i>Draco volans</i>	5	65	Werner, 1922
LARGEST <i>Python molurus</i>	120—60	226,195	
AVES			
SMALLEST Humming Bird	6 (estimated) (shell 14—9.5)	113	
LARGEST Ostrich	50 (shell 155—130)	268,000	
<i>Aepyornis</i> (extinct)	175 (estimated) (shell 37.5 cm 24 cm)	2,806,000	
Hen	40	33,510 (16.17 gms— vitellus) (55.60 gms— whole)	Needham, 1931

TABLE II—*continued*

COMPARATIVE DIMENSIONS OF SOME VERTEBRATE EGGS

Animal.	Diameter of Vitellus (in mm.).	Volume of Vitellus (in cubic mm.).	Authority.
MAMMALIA			
Monotremata			
<i>Ornithorhynchus</i>	2.5 (estimated)	8.2	Hartman, 1929
<i>Echidna</i>	4.4-75 (estimated)	47.7	Flynn & Hill, 1939
Marsupialia			
<i>Dasyurus viverrinus</i>	0.25	0.008	Hill, 1910
<i>Didelphys aurita</i>	0.14-0.16	0.002	Hill, 1918
<i>Didelphys virginiana</i>	0.13 × 0.12	0.001	Hartman, 1916
EUTHERIA			
SMALLEST			
Mouse	0.087	0.000363	Lewis & Wright, 1935
LARGEST			
Sheep	0.15	0.0018	Amoroso, Griffiths & Hamilton, 1942

The egg of the domestic hen has naturally been investigated more fully than that of any other type. The vitellus constitutes the great mass of the ovum; the cytoplasm, which becomes the germinal disc, forms a small cap on top of this enormous mass of yolk (Fig. 14. 3). The yolk is not homogeneous, but consists of a central core of "white" or milky yolk surrounded by concentric layers of yellow yolk, containing lipochrome pigment. The white yolk is mainly situated in the centre of the egg, but it extends upwards, as the latebra, to the germinal disc (Fig. 14. 3). A thin layer of white yolk lies subjacent to the vitelline membrane. The yolk is arranged in the form of spheres with highly refractile granules. The spheres in white yolk vary from 4-75 μ , and in yellow yolk from 25-100 μ .

The white yolk approximates in constitution to that of invertebrate eggs with holoblastic cleavage. It contains twice as much protein as fat, whereas the yellow yolk has twice as much fat as protein. Further, white yolk contains almost twice as much water as yellow yolk and in this respect resembles the albumen. The white yolk is used during early development, with the result that the early embryo derives its metabolic energy and its substance more from protein than from fat.

An analysis of yolk shows it to contain proteins (vitellin, livetin and vitellomucoid), fats (mainly neutral), lecithin and other phospholipins, cholesterol and other steroids, carbohydrates, inorganic salts and a small quantity of haematogen, an iron-containing compound presumed to be the mother substance of haemoglobin.

In the eggs of nidicolous birds the yolk constitutes a smaller proportion of material inside the egg than in nidifugous types and contains 10% more water. For details of the chemical composition of the yolk in different species the reader is referred to Needham, 1931 and 1942.

In birds, yolk is the main source of fat for the future embryo, while albumen is the main source of the protein. The fat and the protein are present in approximately equal proportions. This is in contrast to the egg of fishes where protein predominates (*see* p. 8). More fat is stored in the avian egg than in those of any other form. The large amount of yolk mechanically retards development at the early stage, but eventually it accelerates development, as a larval stage is not necessary.

Mammalia

With the exception of the megalecithal eggs of the monotremes the diameters of the vitellus of the ova of mammals at the time of ovulation (intra-zonal diameters), fall within the size ranges of $87\cdot8\ \mu$ (mouse; Lewis and Wright, 1935) and $250\ \mu$ (*Dasyurus*; Hill, 1910). In eutherian eggs the diameters are usually less than $150\ \mu$. With the completion of the second maturation division and the expulsion of the second polar body, the actual diameter of the cytoplasm, or vitellus, diminishes, but the intra-zonal measurement is not appreciably affected. As Lewis and Wright (1935) and Hamilton and Laing (1946) have pointed out, the volume differences of the eggs are much more striking than are those of the diameters (*see* Table II), but as Hartman (1929) has stressed, the egg of the whale is only a little larger than that of the mouse!

There are marked differences in the appearance of the cytoplasm of the living eggs of different mammals. Even among the eggs of the different species within a single genus, striking differences are apparent (Fig. 14. 8). Certain other differences become apparent after fertilisation (*see* later). In some living eggs, e.g. those of the mouse (Lewis and Wright, 1935) and the golden hamster, the vitellus contains a yellowish granular material, which partly obscures the nucleus. Amongst these granules, and scattered uniformly throughout the cytoplasm, are small clear yolk globules. In the eggs of both these rodents the yolk globules have been demonstrated to be non-fatty in nature (mouse, Gresson, 1933 and 1940; and hamster, personal observations). In other living mammalian eggs, e.g. those of the dog, ferret and horse, the cytoplasm is loaded with fatty and highly refractile globules, which are so dense that the vitellus appears as a dark mass (Fig. 14. 5). In the cow, sheep and goat these fatty globules are also present, but are not so numerous. In the living eggs of the guinea-pig, fatty globules are present, but they are scattered through the cytoplasm (Squier, 1932) and do not show so strikingly as in the types discussed above. In the monkey, as has been demonstrated by Lewis and Hartman (1941), the cytoplasm of fertilised and unfertilised eggs has uniformly distributed fine yolk granules. Lewis and Hartman have further recorded (1933 and 1941) that there are changes in the distribution of the finely granular material, as a result of fertilisation. This, they believe, forms the centrosphere material. Lewis and Wright (1935) have described similar material in the fertilised egg of the mouse. The cytoplasm of the fertilised and unfertilised human ovum has been discussed by Allen *et al.*, 1930, Lewis, 1931, Pincus and Saunders, 1937, Rock and Hertig, 1944, and by Hamilton, 1944 (Fig. 14. 4).

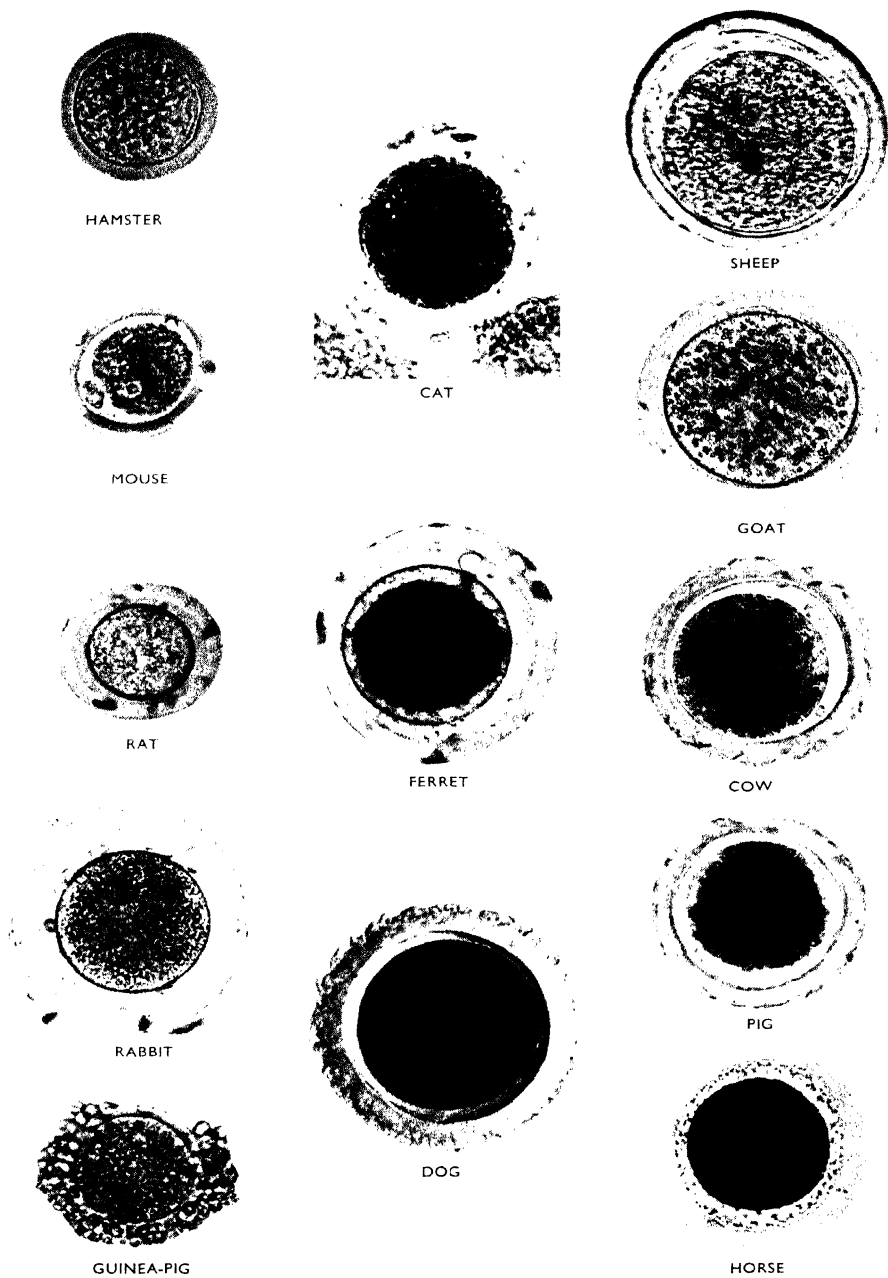


Fig. 14. 5.—Recently ovulated unfertilised living mammalian eggs showing variations in the amount and distribution of deutoplasm. $\times 185$. (The photographs of the eggs of the cat and dog are reproduced by the courtesy of Professor E. C. Amoroso.)

The distribution of mitochondria in mammalian eggs also shows variation from species to species. In the guinea-pig egg, Lams (1913) has shown that the mitochondria are scattered throughout the cytoplasm. In the bat (Levi, 1915 and Van der Stricht, 1923) and in the cat and dog (Van der Stricht, 1923), the mitochondria tend to be concentrated in the cortical zone of the egg. In the rabbit egg (Nihoul, 1927), the mitochondria at first also appear to be concentrated in the peripheral cytoplasm, but migrate to the pronuclear region after fertilisation. A similar phenomenon has been described for the mouse (Gresson, 1941) and appears to be present in the hamster. Aykroyd (1941) has studied the effects of ultra-centrifuging the human ovary. She describes the stratification of the cytoplasm of the more mature oocytes, into two distinct layers. Coagulated cytoplasm occupies more than half of the oocyte, while the Golgi bodies, mitochondria and nucleus are found in the other half. Gresson (1940) has also studied the effects of ultra-centrifuging the mouse ovary. He found that the vitellus of the centrifuged mature oocyte is stratified into four layers: (1) a lightly stained centripetal layer, (2) a layer of yolk, (3) the Golgi layer and (4) a centrifugal layer of mitochondria, which contains the heavy nucleus.

In some eggs, (e.g. those of the guinea-pig, Lams, 1913) the fat is arranged in such a way that they exhibit a distinct polarity. Such polarity is also shown, though not so strikingly in the ova of the mouse (Lams and Doorme,¹ 1907), and the cat (R. van der Stricht, 1911 and Hill and Tribe, 1924), the bat (Van Beneden, 1911), the dog (O. Van der Stricht, 1923), the monkey (Allen, 1928) and the ferret (Hamilton, 1934). Polarity is not recognisable in the ovum of the rat (Sobotta and Burckhard, 1911), the pig (Heuser and Streeter, 1929), the rabbit (Gregory, 1930), the cow (Hartman *et al.* 1931 and Hamilton and Laing, 1946), the goat (Amoroso *et al.* 1942), the horse (Hamilton and Day, 1945), man (Hamilton, 1944) and the sheep (Clark, 1934 and personal observations).

From a study of the literature, it would appear that it is difficult to demonstrate polarity in eutherian eggs which contain an abundance of fatty material, and in those with little fat, but polarity can easily be demonstrated in eggs of the intermediate type.

IV. EGG MEMBRANES

In addition to the differences due to the amount of yolk the eggs of various animal groups show differences in the number and structure of the enclosing envelopes or membranes. These egg membranes, which are not to be confused with the embryonic and foetal membranes of later stages, are additional to the cortical layer of cytoplasm or plasma membrane, which can be demonstrated in all eggs. They are classified, on the basis of their origin, into three types: (1) *primary* egg membranes, formed by the cytoplasm or vitellus of the egg itself; (2) *secondary* egg membranes, produced by the follicle cells which surround the egg in the ovary; and (3) *tertiary* egg membranes, produced by the secretory activity of the lining of the oviduct or the uterus. Tertiary membranes are usually deposited after fertilisation of the ovum. (See Studnička, 1950, for discussion.)

All animal eggs possess one or more of these membranes, but there is a wide range of variations associated with, and appearing to be adaptations for, different

¹ Gresson, however, finds no marked polarity in the egg of the mouse.

environments in early embryonic life. The variations also appear to be associated, to some extent, with difference in the rate of development of the eggs. Eggs deposited in water are usually produced in large number, and are scantily provided with egg membranes. Eggs which require a long time for development outside the maternal body (e.g. those of birds), or those which are exposed to unfavourable conditions during hatching (e.g. deserticolous types), have more and thicker membranes than those deposited in water.

Primary membranes

In most, if not all, eggs there is a vitelline membrane which is a cortical differentiation of the ovarian cytoplasm. Owing to the fact that, in many types, it is more readily visible after fertilisation it is also called the fertilisation membrane. In such types the obvious presence of this membrane is an index that fertilisation has occurred (*see* Chap. 8). The vitelline membrane is usually transparent and apparently structureless. In birds, where it forms the limiting membrane of the yolk (Fig. 14. 3) and performs important physiological activities (*see* Needham, 1942), it is composed mainly of keratin with some intermixed mucoprotein. There is some confusion about the use of the term vitelline membrane, as it has occasionally been used for membranes which are now known to be derived from ovarian follicular cells. To prevent confusion, it is important to restrict the use of the term to the primary membrane.

A further membrane which has a wide distribution amongst vertebrate eggs, is the zona radiata. In some types, (e.g., sharks) it develops inside the vitelline membrane and is therefore to be regarded as a primary membrane. When the zona radiata is outside the vitelline membrane, as in many fishes, amphibians and reptiles, there is some doubt as to its origin. The work of Eigenmann (1890) on *Fundulus* and of Retzius (1912) on *Lacerta viridis*, appears to demonstrate that at least in these types it is a modification of the vitelline membrane and is therefore primary.

Secondary membranes

When present, these are formed by the follicular cells of the ovary which surround the ovum. Secondary membranes are well developed in many of the lower vertebrates where they may form either thick membranes, as in *Myxine*, or thin, as in *Rana*. In mammals the secondary membranes are represented by the zona pellucida which is present in all mammalian eggs, with the possible exceptions of *Elephantulus* (Van der Horst, 1942), *Tarsius* (Hubrecht, 1902) and *Vulpes* (Pearson and Enders, 1943), until they have reached the uterus. Whatever its function, the work of Nicholas and Hall (1942) on the ova of rats, suggests that it is not essential for development. It is a homogeneous, apparently structureless, semi-permeable membrane through which the sperm can readily pass. In some mammals the zona pellucida is readily dissolved by certain fixatives, a fact that has led to erroneous conclusions as to the normal time of its disappearance (*see* Huber, 1915). In pseudo-pregnant rats Blandau (1943) recovered fragmenting ova with intact zonae from the uterine horns as late as 6 days after ovulation. He suggests that the zona pellucida disappears after this time owing to its solution by the increased acidity of the uterine secretions which accompanies the onset of deciduomata

formation. Defrise (1933) and Hall (1936) had previously described the disappearance of the zona and the latter worker had associated the disappearance with increased acidity within the uterus. Fawcett *et al.* (1947), however, record that ova transplanted to extrauterine sites lose their zona pellucida independently of enzymes or other specific conditions of endometrial origin. The zona pellucida of eutherian mammals appears to be moderately resistant to pressure and can be indented without rupture. Its elasticity permits it to become stretched and thinned when the pressure is increased as the morula becomes converted into a blastocyst. Interesting observations on its reactions to pressure and trauma are recorded by Nicholas and Hall (1942). In fixed and stained material the zona pellucida frequently shows a striated appearance in its outer part (Mainland, 1932). Most modern authorities agree that the zona pellucida is solely a product of the follicular epithelium (Schroeder, 1930; Corner, 1932; Flynn and Hill, 1939). Formerly, however, it was considered to be derived from both the ovum itself and the follicular epithelium (Mjassojedoff, 1923). In the bat *Myotis lucifugus* (Guthrie and Jeffers, 1938) it is stated to be of ovarian origin. Here it first becomes visible as a thin acidophilic membrane in the late unilaminar and early bilaminar follicle, and increases rapidly in width to become very distinct in the late secondary follicle. In the eutherian ovarian egg the zona pellucida is closely applied to the underlying vitelline membrane, but after ovulation and fertilisation these membranes become separated by the appearance of the perivitelline space (Fig. 14. 5). Some of the follicular cells which surround the zona pellucida in the follicle persist for a variable time after ovulation as the corona radiata (*see* p. 17).

Flynn and Hill (1939) have shown conclusively that in the monotreme ovarian egg two layers are present between the follicular epithelium and the vitelline membrane—an outer homogeneous layer to which they restrict the term zona pellucida and an inner radially striated layer which they term the striate layer. They reserve their opinion as to the origin of the striate layer, but it seems reasonable to conclude that it is a secondary membrane representing a modification of the inner part of the zona pellucida. Many reptiles and birds also possess the striate layer and hence in this respect the monotreme oocyte agrees with those of Sauropsida.

Tertiary membranes

Unlike the primary and secondary membranes these are formed after ovulation, being secreted by glandular cells in the oviduct or uterus. They may be albuminous and calcareous as in birds, albuminous or mucoproteinous as in a few mammals (e.g. the rabbit), mucinous as in Amphibia, chitinous as in many invertebrates and certain selachians, or keratinous as in monotremes and certain reptiles. The tertiary membranes serve as protective mechanisms for the developing embryo. Some of them, however, of which the most obvious is the albumen, also constitute an important reserve of nutritive material, electrolytes and water. Even the calcareous shell of the avian egg is an important source of calcium for embryonic ossification.

Birds possess a series of tertiary membranes, the hen's egg being the paradigm. Here a spirally wound layer of egg albumen is deposited on the outer surface of the vitelline membrane. The extremities of this layer form the so-called chalazae. Superficial to this layer another, untwisted, layer of egg albumen is deposited.

Round this is laid down the double shell membrane composed of keratin. The two layers of this membrane are separated at the blunt end of the egg to form the air chamber. On the outer surface a porous calcareous shell is deposited (Fig. 14. 3). Finally a very thin protein cuticle covers the outer surface of the shell. Reptilian eggs are very similar to those of birds but chalazae are absent. The "shells" of lizards and snakes are parchment-like, with little calcareous impregnation. Those of crocodiles and some turtles are similar to the shell of the avian egg. In some reptiles, e.g. the tiny lizard *Sphaerodactylus*, the egg shell is soft during laying and hardens later (Klingel, 1944). The chemical and physical properties of the tertiary egg membranes in the different members of the Sauropsida have been little investigated with the exception, of course, of the hen's egg. A cursory investigation, however, shows marked variations. Thus in the eggs of most nidifugous birds the albumen coagulates on boiling, whereas, in most reptiles and nidicolous birds it remains liquid and transparent.

Amongst the mammals tertiary membranes are well developed in the monotremes, which possess an albuminous coat, a shell membrane and modified shell (Hill, 1933, and Flynn and Hill, 1939).¹ In many marsupials (Hartman, 1916, Hill, 1910 and 1918, McCrady, 1938) a relatively thick (0.25 mm.) albuminous coat is laid down on the zona pellucida as the egg passes through the oviduct and a very thin shell membrane (0.001 mm. in thickness, McCrady, 1938) and shell are deposited on the surface of the albumen in the lower part of the tube. In the opossum the shell has a leathery appearance. According to its staining reactions McCrady (1938) has shown that it is composed of an outer, clear homogeneous, and non-chromatophilic matrix within which is a granular basophilic layer.

In eutherian mammals no shell has been recorded, but in a few representatives of this group (notably the rabbit, Fig. 14. 5) there is an extensive layer of albumen or mucoprotein deposited round the ovum in the uterine tube, and this can be regarded as a tertiary membrane. It has been suggested, that the presence of this albumen on the ova of the rabbit a short time after they have entered the uterine tube, is the explanation for the brevity of the period of fertilisability in this species—the sperms being unable to penetrate this tertiary membrane (Hammond, 1934).

Corona radiata. The corona radiata cells are attached to the outer surface of the zona pellucida in ovulating and recently ovulated mammalian eggs. The number of corona radiata cells present varies considerably in different eggs in the same species and the eggs of different species. The length of time that these cells persist after ovulation also shows some variation.

In the egg of the macaque monkey, Lewis and Hartman (1941) have described a marked loosening of the cumulus cells in the pre-ovulatory stages. Amongst the

¹ In Caldwell's (1887) account of the mature ovarian egg of *Ornithorhynchus* he described a layer of "pro-albumen" lying between the follicular epithelium and the zona pellucida. As his term for it suggests, he considered that this layer contributed the albuminous coat to the egg. Hill and his collaborators, however, have shown conclusively that the albumen of the monotreme egg, like that of Sauropsida, is a secretory product of the oviduct. Flynn and Hill (1939) have further suggested that the ovarian "pro-albumen" of Caldwell is equivalent to the follicular fluid of other mammals. They point out that the existence of this "liquor folliculi" in monotremes renders untenable the prevalent view that the monotreme follicle differs from that of all other mammals and agrees with those of reptiles and birds in being solid throughout and devoid of any trace of follicular fluid.

ungulates, the corona radiata cells disappear soon after ovulation. The absence of these cells has been recorded for the roe deer (Bischoff, 1854), the sheep (Assheton, 1898 ; McKenzie and Allen, 1933 ; Clark, 1934, and McKenzie and Terrill, 1937), the cow (Hartman *et al.*, 1931 ; Evans and Miller, 1935, and Hamilton and Laing, 1946), the pig (Assheton, 1898b, Corner and Amsbaugh, 1917, and Heuser and Streeter, 1929) and the horse (Amoroso *et al.*, 1939 and Hamilton and Day, 1945).

For some time after ovulation a well developed corona radiata is found with regularity in the rabbit (Gregory, 1930, and Pincus and Enzmann, 1932), the guinea-pig (Squier, 1932), the rat (Gilchrist and Pincus, 1932), the mouse (Lewis and Wright, 1935), the cat (Hill and Tribe, 1924), the dog (Evans and Cole, 1931), the ferret (Hamilton, 1934) and man (Allen *et al.*, 1930, and Hamilton, 1944). McCrady (1938) has shown that the corona cells soon disappear in the opossum. In the opossum the ova pass through the uterine tube in 24 hours, whereas in higher mammals, the approximate length of time involved is $3\frac{1}{2}$ days.

The dissolution of the corona radiata cells seems to involve a definite process in the tubes and may be brought about by changes within the cells themselves. In some animals, e.g. the guinea-pig (Squier, 1932), the cells are firmly attached to the zona pellucida and cannot be completely removed by dissection. In the unfertilised eggs of most mammals, the corona cells show degenerative changes when they reach the distal portion of the tube. There can be little doubt that the presence of living spermatozoa may assist in the separation of the cells. That they are not essential, however, is shown by the fact that the corona cells disappear in unfertilised as well as in fertilised eggs. Pincus (1930) and Pincus and Enzmann (1932) have shown in the rabbit that when eggs with attached cumulus cells are placed *in vitro* with living sperm suspensions, the cells are rapidly dispersed, but that this does not occur in sperm-free media. Further details are given in a combined paper (Pincus and Enzmann, 1935). Yamane (1930 and 1935) believes that the dispersion is due to a proteolytic enzyme in the spermatozoa. For the details of the effects of sperm suspension on maturation, the work of Pincus and Enzmann (1935) should be consulted. Swyer (1947) has shown that the disintegration of the corona radiata from the rabbit's ovum is not affected by hyaluronidase, but by some factor produced by the uterine tube.

McClellan and Rowlands (1942) have suggested that the enzyme is a hyaluronidase similar to, if not identical with the "spreading factor" of testicular extracts. They have suggested that the large number of spermatozoa required to ensure fertilisation of an ovum may possibly be due to the necessity of achieving a concentration of hyaluronidase sufficient to liquefy the hyaluronic acid gel and so allow penetration of the egg by the effective sperm. These workers also studied the effects of certain detergents on the corona radiata. More recently, Rowlands (1944) has reported preliminary work on the effects of adding hyaluronidase to concentrations of sperm which alone would be insufficient to cause fertilisation. The experiments suggest that the enzyme does, in fact, facilitate fertilisation by sperms in what would normally be an insufficient concentration. It must be pointed out, however, that the cumulus is not invariably present and the hyaluronidase may be acting on the zona pellucida or the vitelline membrane. Pincus (1939a), however, has shown that heterologous sperms do not usually perforate the zona.

V. STAGES OF DEVELOPMENT

Although normal development is a continuous process it is convenient for descriptive purposes to divide it into stages. In early development the successive stages are *maturation*, *fertilisation*, *cleavage*, *blastula formation*, *gastrulation* and, finally, one known, in Amphibia, as the *neurula*. This last stage corresponds more or less to the somite stage in mammalian development and when it is completed the general embryonic pattern is well established. As there are no specific organs for the performance of specific functions present during the above-named stages of development, they are often taken collectively to constitute the *pre-functional period* of development. Stages succeeding that of the *neurula* are then said to be in the *functional period* of development.

The initial processes of development in different animal groups show certain common fundamental characters especially in the growth and maturation of the egg and the accompanying nuclear changes (*see* Chap. 5). Fertilisation tends to be a little more specialised in the different groups, but even here the variations are not considerable (*see* Chap. 8). Maturation and fertilisation are followed by the phenomenon of *cleavage*.¹

Cleavage

Cleavage is a special case of mitotic division and a detailed study of its mechanism must necessarily include a review of knowledge on mitotic division in general. Such a review is beyond the scope of the present survey. As cleavage is an important developmental process, however, a brief account of some of its salient physiological and cytological features will be presented (p. 34) after the process has been described in the different vertebrate types.

Cleavage consists of a rapid succession of mitotic divisions of the fertilised ovum or zygote. It results in the production of a progressively increasing number of small cells, called blastomeres (Figs. 14. 1 and 9). Parthenogenetic cleavage may occur in the absence of fertilisation (*see* p. 41). During cleavage there is little or no increase in the amount of protoplasm. Indeed in many cases (e.g. in the eutherian mammalian egg) there appears to be an actual diminution of the protoplasmic mass. Cleavage produces three main effects. In the first place it results in the partitioning of the material of the zygote, usually more or less equally, at other times unequally, amongst the resulting blastomeres (Fig. 14. 2). Secondly, the formation, during cleavage, of separate blastomeres gives increased freedom of movement to the original protoplasmic mass and facilitates the important morphogenetic movements of later development. Finally, since eggs are very large, when compared with the usual somatic cell size of the animals from which they have been derived, the process of cleavage results in the production of increasing numbers of progressively smaller cells each of which gradually approximates to the somatic size. Whitaker (1933) has suggested that owing to its large size the ovum is in an abnormal state of metabolism until fertilisation. Subsequently, as cleavage commences and progresses, more normal cellular metabolic conditions are increasingly restored as the individual cells diminish to the normal size. Berrill

¹ Cleavage is sometimes referred to as segmentation but owing to the fact that the latter term is also used for quite a different process in later development, its use in the present context has been avoided.

(1945) has indicated that from this viewpoint cleavage can be regarded primarily as an automatic re-establishment of normal cell sizes. Cleavage rate diminishes with the decreasing cell size until it ceases with the attainment of the minimum cell sizes typical of the species. The number of cells finally produced depends upon the cell size characteristic of the species, the size of the egg and the amount and nature of its deutoplasmic reserves. Thus the eggs of *Ascidia* and *Amphioxus* are similar in size and yolk content but as the cell-sizes finally attained are different, cleavage in *Ascidia* results in some 3,000 cells while in *Amphioxus* it produces about 9,000 cells.

Cleavage can be classified in several different ways. Thus it can be considered on the basis of the qualitative partitioning of the zygote, so that different blastomeres are related to specific regions in later developmental stages. Again it can be classified according to the actual pattern of the divisions, this pattern being largely dependent on the amount and distribution of the stored materials in the mature egg. Neither method of classification of cleavage is absolute since transitional types are shown in each.

Using the first of these methods, cleavage is divided into two principal types: *determinate* and *indeterminate*. Determinate cleavage occurs in the so-called mosaic, or pre-delineated, eggs of a number of invertebrates, e.g. annelids, ascidians and mollusca (with the exception of cephalopods). In such eggs, often before cleavage, definite organ-forming regions or areas can be recognised. The study of the history of the individual blastomeres (cell lineage) of these organisms shows that the organ-forming regions, and the cells derived from them, produce definite and specific organs in later development. That is to say, the different portions of the mature egg are destined for a particular fate in normal development and each portion is capable of producing only specific organ regions or tissue.

Indeterminate cleavage occurs in eggs which are called "regulative." Organ-forming regions cannot be identified in the mature ovum and differentiation occurs relatively late. Only after the egg consists of many blastomeres, all of which appear alike, can presumptive organs be identified. Further, up to quite a late stage of cleavage (or even later) blastomeres and embryonic cells are pluripotent and possess the power of adapting themselves to the cellular environment in which they are situated or placed. It is for this reason that eggs showing this type of cleavage are often called "regulative" eggs. They are found in chordates, cephalopod molluscs, most arthropods and echinoderms and some insects.

The concept of two opposed types of development which is implied by the classification of eggs into those of the "mosaic" type, with determinate cleavage, and those of the "regulative" type, with indeterminate cleavage, has had an important influence on the history of experimental embryology. Much modern work, however, has shown that all eggs are capable of some degree of regulation and that there is a whole range of intermediate types between markedly "mosaic" and freely regulative eggs (for discussion see Dalcq, 1941, and J. Brachet, 1944).

The other customary classification of cleavage is on the basis of the actual pattern of the cell division; here again cleavage can be divided into two main types, *holoblastic* and *meroblastic*. In holoblastic (also known as complete and equal) cleavage the whole zygote divides at first into two, and later by successive dichotomous division into a number of approximately equal blastomeres (Fig. 14. 13).

This pattern of cleavage is found in miolecithal and medialecithal eggs (*see* Table I). In meroblastic (incomplete, partial or discoidal) cleavage, which occurs in megalecithal eggs (Table I), only the protoplasmic (or "animal") pole undergoes division as the large amount of deutoplasm inhibits the division of the "vegetal" pole (Fig. 14. 20). Here also there are intermediate and transitional varieties of eggs and cleavage patterns which do not conform to either of the main varieties.

Cleavage in infra-mammalian vertebrate eggs

In the phylum Chordata, miolecithal, medialecithal and megalecithal eggs are all found although the latter two varieties are more common. Consequently both holoblastic and meroblastic varieties of cleavage are found.

Miolecithal eggs.—These are found in *Amphioxus* and in the Tunicata (with the exception of the family Pyrosomidae where they are megalecithal). As a classical subject for embryological investigation the early development of *Amphioxus* has been widely studied. The following account of cleavage of the egg of this acraniate is based on the careful work of Conklin (1932). After fertilisation the pronuclei and the first cleavage spindle lie in an area of hyaloplasm which is situated slightly eccentrically and nearer the animal pole. Conklin has demonstrated that in the cytoplasm of the egg after fertilisation the hyaloplasm has the form of a hemispherical shell and is separated from the surface of the egg antero-dorsally by a thick mass of yolk which is the presumptive endodermal area of the future embryo. Postero-ventrally the hyaloplasm is separated from the surface by a thin layer of yolk spherules—from this region the future notochord and ectoderm will be derived. That part of the cytoplasm which will give origin to the future mesoderm is crescentic in shape and lies in the path of entry of the sperm, posterior to the presumptive endoderm. As a result of this arrangement of the cytoplasm at the time of pronuclear conjugation the unsegmented fertilised egg of *Amphioxus* is bilaterally symmetrical. Further, as indicated above, the structure of the cytoplasm is such that the location of the three germinal layers of subsequent development is already foreshadowed. The first cleavage, which is meridional, divides the egg into a right and a left blastomere of approximately equal size. The second cleavage, also meridional and at right angles to the first, divides the egg into two slightly larger anterior and two smaller posterior blastomeres. The latter contain the mesodermal crescent. The third division is nearly equatorial and results in the formation of eight blastomeres, of which the four (micromeres) at the "animal" pole are slightly smaller than the four (macromeres) at the "vegetal" pole. Successive division results in the formation of a morula with smaller cells at the animal pole and larger cells at the vegetal pole (Fig. 14. 1). With the completion of the eighth cleavage, the cells of the morula become arranged to form a hollow sphere, the blastula. Synchronous cleavage has now come to an end. The cavity (blastocoele) of the blastula is surrounded by cells which are slightly larger at the vegetal pole (presumptive endoderm) and slightly smaller at the animal pole (presumptive ectoderm and chorda, Fig. 14. 18A). The presumptive mesoderm is represented by a crescent of small rapidly dividing cells which surrounds the presumptive endoderm on its posterior and lateral border (Fig. 14. 18A). During cleavage in *Amphioxus* a transparent jelly-like substance is found between the blastomeres. Later the jelly is found in the blastocoele where its swelling leads to enlargement of the cavity.

Medialecithal eggs.—These are found in Amphibia (with the exception of Apoda), Petromyzontidae, Chondrostei, Amiidae, Holocephali and Dipnoi. As these eggs possess only a moderate amount of yolk the first two cleavages, which are meridional, normally result in four equal blastomeres. The third cleavage is equatorial and cuts off animal pole cells which are much smaller than those at the vegetal pole (holoblastic and *unequal* cleavage). The animal pole cells contain much less yolk than those of the vegetal pole and their division rate is much more rapid. They play the predominant part in the formation of the embryo. In such eggs the blastocoele is relatively small, and is placed eccentrically nearer the animal pole (Fig. 14. 2).

During cleavage in the amphibian egg there are no spectacular changes in its different cytoplasmic regions. Staining experiments by Schechtman (1934, 1935, 1937) and by Nicholas (1945), however, demonstrate that there is an early unipolar ingression of materials from the cortical region and vegetal pole elements can be traced to the internal yolk mass, where, in later development, they are found in relation to the blastocoele. There are also marked alterations in the biochemical organisation of the cleaving egg. Thus the basophilia of the cytoplasm is accentuated, the yolk granules become smaller and there is a marked increase in histologically detectable sulphhydryl proteins and ribose nucleoprotein. The nuclei decrease in size as cleavage progresses but their increase in number is such that a marked synthesis of thymonucleic acid obviously must occur. Schonmann (1938) has shown that, in the urodele egg, there is a characteristic decline in nuclear volume after the stage of the young blastula. Hence the nuclei of the later blastula possess only one-eighth of the volume of those of earlier stages. This change is the result of the absence in these later stages of rhythmical nuclear growth during inter-kinesis. J. Brachet (1938) has described a marked increase in the sulphhydryl protein content of the nuclei in the correspondingly late stages, but the relationship of this fact to the decrease in nuclear size is doubtful.

The problem of the attractive forces which bind the cleaving cells together has received much attention. "Sticky substances" possibly play some part as does also thigmotaxis (*see* Holtfreter, 1939, and Lehmann, 1945). When cleaving cells are separated they show no cytotropism, but if they do come into direct contact they again unite to form a unit. The nature of the attractive force ("*Anziehungskraft*") is unknown, but there is no selective attraction of presumptive endoderm cells to presumptive endoderm cells, etc. The existence of this non-selective affinity has made possible germinal fusions and transplants of cleavage and blastula stages. For an appreciation of the complicated environment in which the individual cells of a cleaving egg find themselves the reader is referred to Holtfreter's papers (1943, 1944, 1947a and b) and to Nicholas' stimulating discussion (1945).

Megalecithal eggs.—These eggs are found in reptiles, birds and, amongst the fishes, in Eusalachii and Teleostei. Although the amount of yolk varies considerably in different types, there is, in all of them, enough deutoplasm to limit cleavage to the meroblastic or incomplete method. The cytoplasm, in which the nucleus is situated, is always an inconspicuous and relatively minute mass at the animal pole. Cleavage in such eggs involves only this cytoplasmic region and is at first simply a furrowing of the cytoplasm. It will be realised that such cleavage furrows merely delimit the cell areas of prospective blastomeres whose deeper

surfaces are continuous with each other and the underlying yolk. There is much variation in the details of the cleavage pattern in the different varieties of megalecithal eggs, but the resulting blastula is always a flattened disc of cells, the blastodisc. This rests on the mass of yolk, but is apparently separated from it, except at the blastodisc margin, by a shallow cleft-like space—the “sub-germinal cavity” (Figs. 14, 20). In many megalecithal eggs after cleavage an intermediate layer is found under the blastodisc. This layer contains numerous nuclei, but is not divided into cells. It is the vitelline syncytium and may be regarded as an organ of digestion for the transference of yolk to the actively dividing cells of the blastocyst. Cordier (1941) has suggested that the vitelline syncytium behaves as a dialysing membrane and J. Brachet (1941) has pointed out that it is possible that hydrolysis of the deutoplasm occurs in the syncytial region as van der Ghinst (1934) has demonstrated enzymes in it.

Dalcq and Pasteels (1938) and Pasteels (1940, 1945) consider that the “sub-germinal cavity” of sauropsidan development is not to be regarded as a blastocoele. Pasteels (1945), indeed, states “that the ‘cavity’ is, in fact, only the result of a progressive liquefaction of the yolk acted upon by enzymes produced by the yolk syncytium and perhaps by the blastoderm itself.” As this conception is of importance in considering the process of avian gastrulation and endoderm formation it is again discussed later (p. 55).

Cleavage in mammals

Apart from the monotremes all mammals have miolecithal eggs which undergo holoblastic and almost equal cleavage. The miolecithal condition in metatheria and eutheria, however, is generally believed to represent a reversal in evolution, for the eggs of the ancestral oviparous mammal-like reptiles were probably megalecithal. Further, although they are highly specialised, the prototheria, possessing megalecithal ova with meroblastic cleavage, are annectant to the primitive mammals. Finally, many of the features of the early development of the marsupials and placental mammals suggest that there has been a loss of yolk from the egg in the course of evolution.

Prototheria.—Caldwell in 1884 and 1887, definitely established the existence of oviparity in the monotremes and that the cleavage of their eggs is meroblastic. His work and the fuller investigations of Semon (1894), Wilson and Hill (1905), and Flynn and Hill (1939, 1942 and 1947) have demonstrated that, in essential details, the processes of ovarian maturation, fertilisation and early cleavage in this group closely resemble the same processes in Sauropsida.

The final form of the unsegmented, circular blastodisc of the egg of *Echidna* or *Ornithorhynchus*, consists of a superficial zone of granular cytoplasm and an underlying vacuolated zone containing fine yolk spherules in the cytoplasm. When the fertilised blastodisc prepares to cleave, fine, elliptical yolk spheres infiltrate into one of its ends. The conjugating pro-nuclei lie nearer this end and in the long axis of the blastodisc (Fig. 14. 6). Flynn and Hill (1939) state that the male pro-nucleus lies deeper, is smaller and is more darkly stained than the female pro-nucleus, but the evidence upon which they base this assumption does not appear conclusive. Contrary to earlier statements of Gatenby and Hill (1924), the work by Flynn and Hill seems definitely to establish that polyspermy does not normally occur in monotremes.

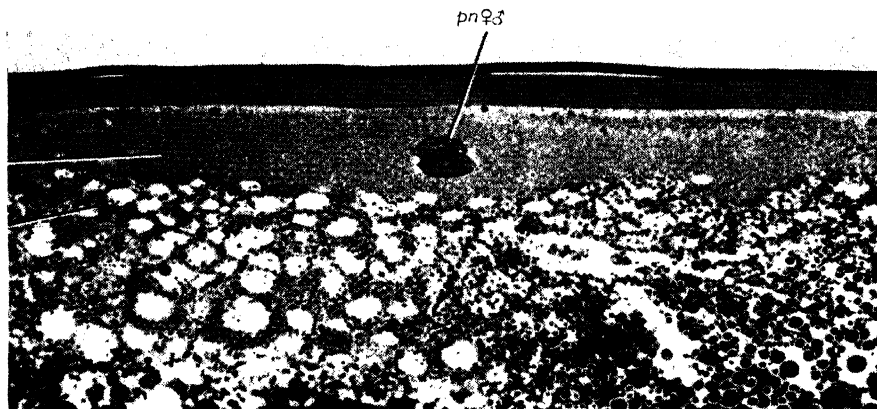


Fig. 14. 6---A section of *Echidna* egg to show germinal disc. The male and female pro-nuclei are seen overlapping each other at the centre of the formative zone of the disc. $\times 400$. (Reproduced by the courtesy of Professors Flynn and Hill, and the Zoological Society.)

Before proceeding to the first cleavage spindle, the pronuclei fuse. Caldwell described the first cleavage furrow as dividing the blastodisc into a larger and a smaller area, the latter containing more yolk than the former. Flynn and Hill's work (1939) has shown that the second cleavage furrow is at right angles to the first, being coincident with the long axis of the elliptical blastodisc. The furrows

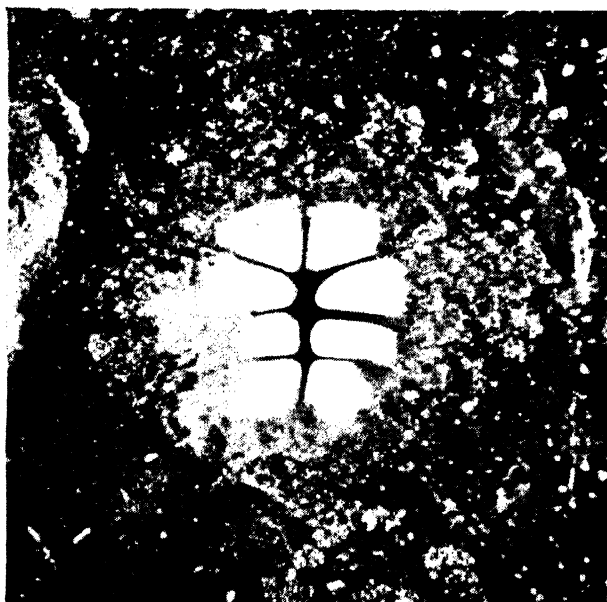


Fig. 14. 7---Surface view of blastocyst of *Ornithorhynchus* egg. $\times 58$. (Reproduced by the courtesy of Professors Flynn and Hill, and the Zoological Society.)

of the third cleavage then appear on either side of the first, and more or less parallel to it. The resulting 8-cell stage (Fig. 14. 7) forms two linear aggregates, each of 4 cells symmetrically arranged on each side of the long axis of the blastodisc. The fourth and fifth series of furrows result in 16 and 32 prospective blastomeres respectively (Figs. 14. 8A and B). Up to the latter stage, synchronisation of division is practically complete, but the prospective blastomeres of this stage consist of an outer marginal ring of larger and an inner central field of smaller blastomeres. In later blastodiscs the division of the blastomeres does not follow any regular plan or pattern and there is often a considerable time lag between the nuclear and cytoplasmic divisions. The centrally placed blastomeres become delimited from

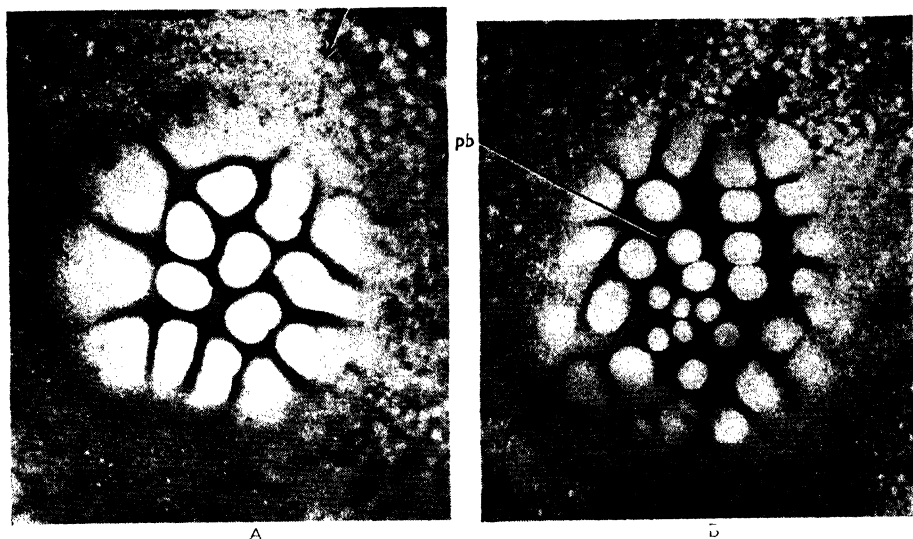


Fig. 14. 8 — A. Surface view of blastodisc of *Echinidna* egg at the 16-celled stage. $\times 60$. B. Surface view of blastodisc of *Echinidna* egg at the 32-celled stage. $\times 62$.
(Reproduced by the courtesy of Professors Flynn and Hill, and the Zoological Society.)

the yolk bed and undergo further division in planes oblique to the surface. The result of this oblique division, is the formation of a superficial layer of larger cells and a deep layer of smaller cells. The marginal cells of the blastodisc also undergo further division, resulting partly in the production of cells contributing to the periphery of the blastodisc and of other cells called vitellocytes by Flynn and Hill. The vitellocytes possess migratory properties, and the capacity of enclosing yolk spheres. They correspond to the so-called periblast of Sauropsida and form a yolk syncytium, which encircles the blastodisc in the form of a continuous zone of nucleated cytoplasm. This zone gradually extends round and encloses the yolk. The peripheral migration of this germinal zone (called the germinal wall by Flynn and Hill) conditions the growth and surface extent of the blastodisc and its conversion from a thick biconvex disc (six to seven cells in thickness) into a thin blastoderm, of only two to three cells in thickness, which follows the germinal margin around to enclose the yolk mass of the egg. The site of closure of the blastoderm is the yolk navel.

This enclosure of the yolk by a complete blastodermic cellular wall marks the establishment, in the monotreme, of that typical mammalian development stage, the blastocyst. The deeply placed cells in the early blastoderm become intercalated amongst the superficial cells as the thin blastodermic membrane is established, so that the latter becomes a unilaminar blastoderm, as originally described by Semon. In this unilaminar blastoderm, two types of cells can be recognised—prospective ectodermal and primitive endodermal cells. Flynn and Hill regard the attainment of the unilaminar condition as heralding the commencing formation of the primary germ layers. The prospective ectodermal cells have reached their definitive position at the surface. The primitive endodermal cells then sort themselves out and later migrate to a deeper position (*see* p. 56).

Metatheria.—Cleavage in marsupials has been described in *Didelphys virginiana* by Selenka (1886), *Dasyurus viverrinus* by Hill (1910), *Didelphys aurita* by Hill (1918), and *Didelphys virginiana* by Hartman (1916 and 1919) and by McCrady (1938). McCrady's paper summarises the earlier literature and the following account is largely based on it.

The pronuclei fuse in approximately the centre of the egg before the formation of the first cleavage spindle which comes to be eccentrically situated. The resulting plane of division is also "off centre," so that when the daughter cells are formed they are unequal in size (in the ratio of 60% to 40%). During the fusion of the pronuclei and the first cleavage, yolk granules and fat droplets of varying size are extruded from the surface of the ovum into the perivitelline space. In *Dasyurus*, Hill (1910) has described the extrusion of the yolk as a large mass from one pole of the ovum. (For further details *see* under Deutoplasmolysis, p. 40.) Already, before the completion of the first cleavage it can be seen that the chromosomes which pass into the presumptive larger blastomere are in a more advanced state than those which pass into the smaller blastomere. This difference is reflected in the subsequent earlier division of the larger blastomere (A) than in the smaller one (B). Before the cleavage furrow has appeared in B, A has divided meridionally so that a three-cell stage composed of A_1 and A_2 and B is formed. Division of B results in a four-cell stage, comprising two larger blastomeres A_1 and A_2 and two smaller ones B_1 and B_2 . In *Didelphys virginiana*, the division spindle of the blastomere B is arranged at right angles to that of A. Consequently, the blastomeres of the four-cell stage are arranged in a cross manner as in eutheria (*see* p. 29). In *Dasyurus viverrinus*, Hill found that the second cleavage division always resulted in a radial arrangement of the four-cell stage. Selenka (1886) also figures the four-cell stage of *Didelphys virginiana* as being radial, but the work of Hartman (1916 and 1919) and Smith (1925) has shown that such radially arranged four-cell stages are always abnormal. In fact, they are usually not cleavage stages at all, but fragmented unfertilised eggs (*see* p. 41). McCrady (1938) interprets the different orientation of the second spindle as a mechanical result of simple pressure relations, induced by the earlier gelation of the spindle in the larger blastomere. By the end of the four-cell stage the total mass of cytoplasm of the blastomeres has been reduced by the elimination of deutoplasm, so that the cells no longer fill the space within the zonal cavity. They are separated from each other and to a lesser extent from the zona pellucida by the extruded deutoplasmic granules. There is little further yolk extrusion after this stage. In subsequent cleavage, the larger cells of the four-cell stage (A pole cells) divide more rapidly than

the smaller cells (B pole cells). The times of division of the individual blastomeres are not synchronous so that stages with from five to thirty-two blastomeres have been found. At the thirty-two-cell stage the blastomeres are arranged against the

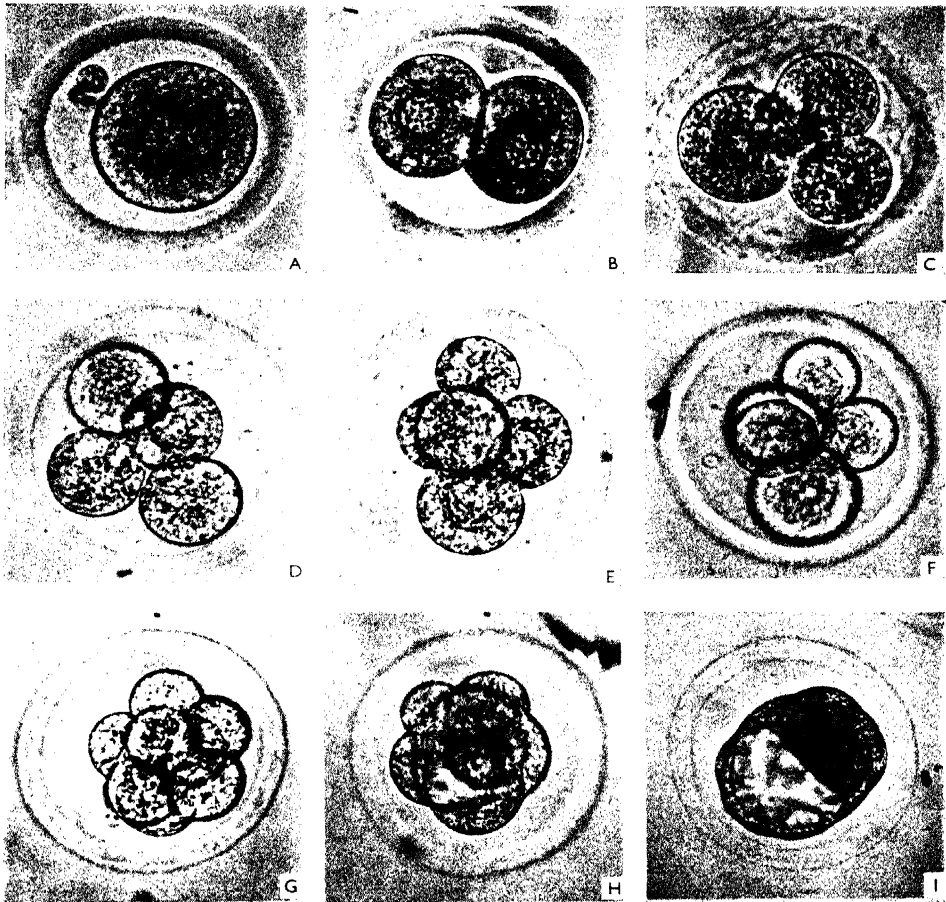


Fig. 14. 9.—Fertilisation and cleavage stages of the living egg of the golden hamster, *Cricetus auratus*. $\times 300$.

A. Centrally placed pronuclei. B. 2-celled stage. C. 3-celled stage. D. 4-celled stage. E. 5-celled stage. F. 6-celled stage. G. 8-celled stage. H. Early blastocyst. I. Later blastocyst.

inner aspect of the zona pellucida and form a unilaminar blastocyst. Identification of individual blastomeres can be followed, according to McCrady, as far as the twelve-cell stage with accuracy; in later stages cell lineage can no longer be established with certainty, although the smaller cells at the so called animal pole are believed to have been derived from the initially larger blastomere (A). The arrangement of the blastomeres against the zona pellucida at the blastocyst stage, is thought by McCrady to be due to stereotropism rather than to endosmosis. Whatever the

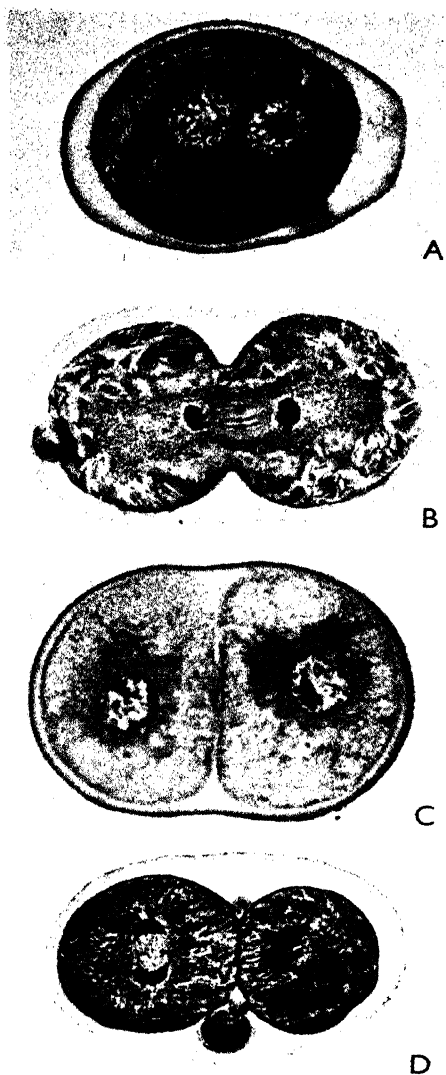


Fig. 14. 10—Sections of cleavage stages of the egg of the golden hamster, *Cricetus auratus*. $\times 640$. A. Centrally placed pro-nuclei. B. First cleavage spindle. C. 2-celled stage. D. Second cleavage spindles at right angles to each other.

explanation of this arrangement may be, the extruded yolk granules are gradually displaced from the zona pellucida by the blastomeres and come passively to occupy the blastocyst cavity. It should be stressed that the total volume of the cytoplasm of the blastomeres has not increased from the four-cell stage. The zona pellucida becomes thinned during cleavage, but the albumen layer and shell membrane are thicker. In *Dasyurus*, Hill states that by the end of the fourth cleavage division the eight blastomeres can be divided into an upper ring of four smaller more transparent cells with relatively little deutoplasm, and a lower ring of four larger more opaque cells with more abundant deutoplasm. Hill believed that the cells derived from the upper ring give origin to the formative embryonal region of the blastocyst wall, while those derived from the lower ring form the extra-embryonic trophoblastic area. (For further discussion of the polarity of the cleaving ovum see p. 14.)

Eutheria.—After fertilisation in eutherian mammals, the pronuclei come together and fuse in approximately the centre of the egg (Figs. 14. 9 and 10). At the time of fusion they are similar in appearance and of equal size. In most eutherian eggs at this stage it is not possible to differentiate the male from the female pronucleus. In some eggs, however, the sperm tail remains attached to the male pronucleus until this has reached its definitive position (mouse, Lams and Doorme, 1907; rabbit, Sobotta and Burckhard, 1911; and guinea-pig, Lams, 1913). After the pronuclei have reached their definitive position, a line joining their centres indicates the plane of the first cleavage. In eggs which

exhibit polarity, this plane passes through the animal and vegetal poles.

The completion of the first cleavage division results in a two-cell stage (Figs. 14. 10 and 11) in which the blastomeres are slightly different in size (see p. 31). The second cleavage divisions are effected in two planes at right angles to each

other, and as the larger blastomere usually divides first, a transitional three-cell stage is frequently found. At the four-cell stage the blastomeres are usually, if not always, arranged in pairs so as to form a cross-shaped grouping, i.e. the four cells form a tetrahedron (Fig. 14. 9). The tetrahedral arrangement of the blastomeres at this stage is due in the first instance to the orientation of the two spindles of the second cleavage at right angles to each other (Fig. 14. 10). Such an orientation has been recorded for a large number of eutherian mammalian types—the rabbit (Assheton, 1894), the monkey (Lewis and Hartman, 1933), the ferret (Hamilton, 1934), the goat (Amoroso *et al.*, 1942), the hamster (unpublished results), and has also been recorded for marsupials (*see* p. 26). The blastomeres undoubtedly occupy less space inside the zona pellucida when arranged in the tetrahedral manner, but it is generally considered that the cross-shaped grouping has a significance other than that of mere packing (*see*, however, van der Horst, 1942).

At the four-cell stage, differences in the size of the blastomeres are frequently found (*see* Heuser and Streeter, 1929, Hamilton, 1934, Amoroso *et al.*, 1942, and Hamilton and Laing, 1946, for reviews on this subject). The third cleavage divisions do not occur synchronously in all the cells of the four-cell stage so that five-, six- and seven-cell stages are frequently found (Fig. 14. 9). At the eight-cell stage, differences in the size of the blastomeres are present in most mammalian eggs examined.

As the fourth cleavage divisions are again not synchronous in all of the blastomeres of the eight-cell stage, intermediate numbers between the eight- and the sixteen-cell stage are commonly found. The cells now show further discrepancies in size and by the time of completion of this cleavage one or more cells are centrally placed. The central cells reach this position as the result of a radial arrangement of one or more spindles of division at the eight-cell stage (but *see* p. 32). The resulting group of cells, called a morula, is characteristic of most eutherian types (Fig. 14. 13). Subsequent cleavage divisions are usually markedly asynchronous with the peripherally situated cells dividing more actively. At the same time fluid collects in the intercellular spaces between the peripheral (trophoblastic) cells and the central (inner cell) mass (*see* p. 33). The resulting small vesicle is called a blastocyst (Figs. 14. 12 and 14). This pattern of cleavage and of morula and blastocyst formation is followed by all eutherian mammals, whose early development has been studied, with the exception of *Hemicentetes* (Goetz, 1937 and 1938) and *Elephantulus* (van der Horst, 1942). In these two insectivores a morular stage is not formed and the blastocyst ("mammalian blastula") which results from cleavage does not initially possess an inner cell mass (Fig. 14. 56) and is very similar to the marsupial blastocyst. The blastocyst in these insectivores, however, does not appear to arise in the same fashion as in the marsupials and it is probably best considered to result from a very precocious appearance of the blastocyst cavity. In *Elephantulus* it is stated to arise as early as the four-cell stage and it is well established in *Hemicentetes* at the sixteen-cell stage.

During cleavage there are noteworthy alterations in the volume of the vitellus. Gilchrist and Pincus (1932) found, in the rat, a shrinkage of from 13 to 17 per cent. in the volume of the vitellus at the time of fertilisation. In *Macacus rhesus*, Lewis and Hartman (1933) found a shrinkage of 44 per cent. as the result of the first cleavage division. During the one-cell stage of the mouse, Lewis and Wright (1935) observed a shrinkage of as much as 25 per cent. with additional diminution

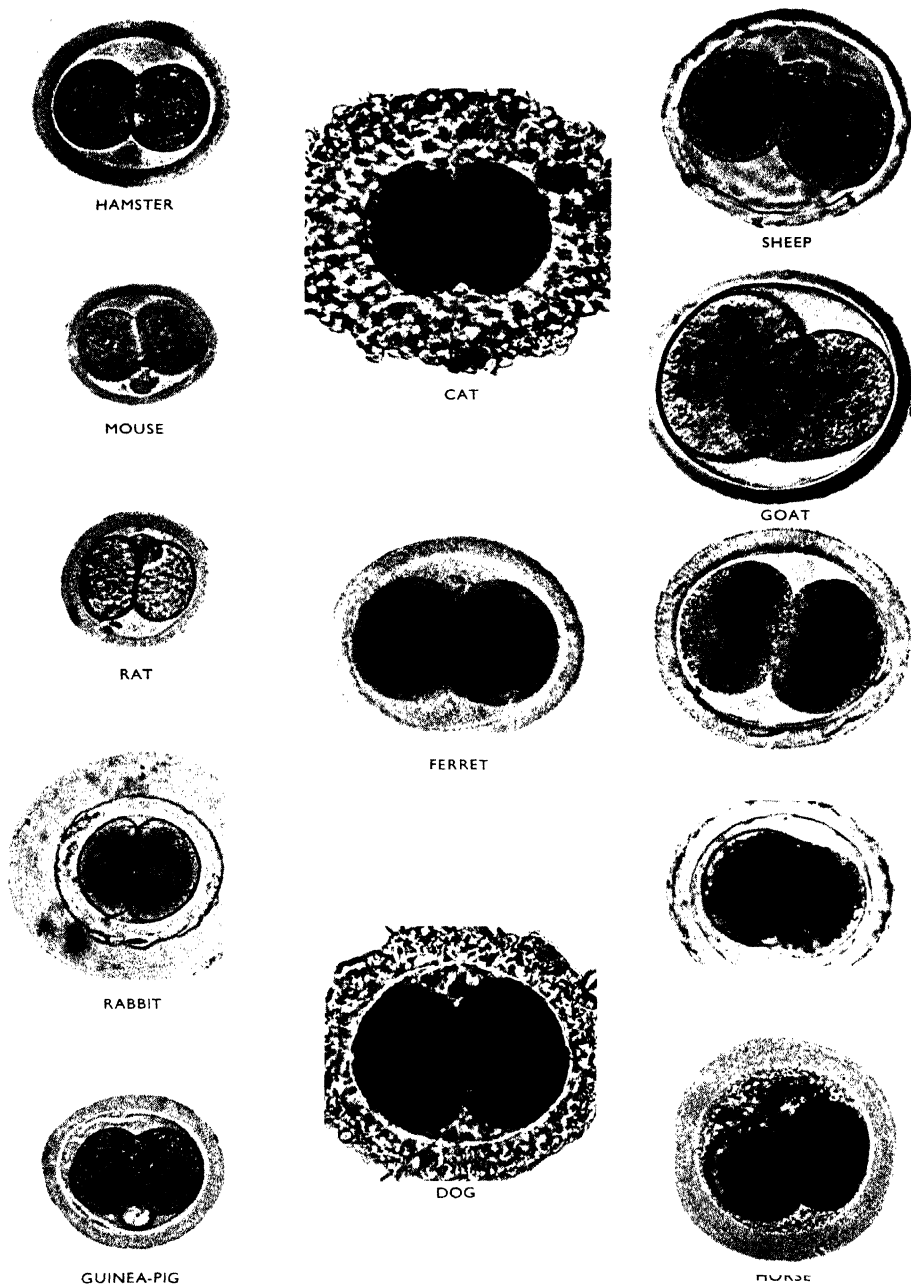


Fig. 14, 11—Living 2-celled stages of mammalian eggs. $\times 185$, except that of the rabbit which is $\times 125$. (The photographs of the eggs of the cat and dog are reproduced by the courtesy of Professor E. C. Amoroso.)

in vitelline volume at the two-celled stage and later. Shrinkage in volume has also been recorded by Hamilton and Day (1945) in the horse; and by Hamilton and Laing (1946) in the cow. It is also considerable in the cleaving eggs of the golden hamster, *Cricetus auratus* (unpublished results).

Cell lineage during cleavage of the eutherian mammalian egg

It is generally believed by mammalian embryologists that, with the techniques hitherto employed, it is neither possible to trace the history of individual blastomeres of early cleavage very far in subsequent development, nor that there is an early determination (or "segregation") of the fate of these early blastomeres. Many conflicting opinions regarding the size, the staining properties and the prospective significance of the first two blastomeres have, however, been expressed. Certainly discrepancies in blastomere size are common, but the work of Hamilton (1934) on the ferret suggests that the size difference can be explained as a chance separation of the cytoplasmic material at the first cleavage. His work, and that of investigators on many other species, does not suggest obvious qualitative differences between the first two blastomeres. Thus Gresson (1941) has shown that in the mouse the mitochondria are equally distributed between the first two blastomeres. A number of distinguished workers in this field of embryology have, however, described or implied such differences (rabbit, van Beneden, 1875; *Tarsius*, Hubrecht, 1902; rat, Huber, 1915; dog, O. van der Stricht, 1923; cat, R. van der Stricht, 1911 and Hill and Tribe, 1924; and mouse, Sobotta, 1924). Van Beneden and Sobotta believed that at the first cleavage the material for the formation of the

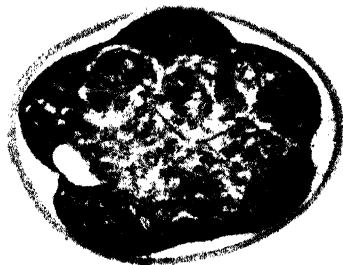


Fig. 14. 12—Section of an early blastocyst of the golden hamster, *Cricetus auratus*. The outer cells are developing to form the trophoblast which is more darkly stained than the centrally-placed cells. $\times 640$.

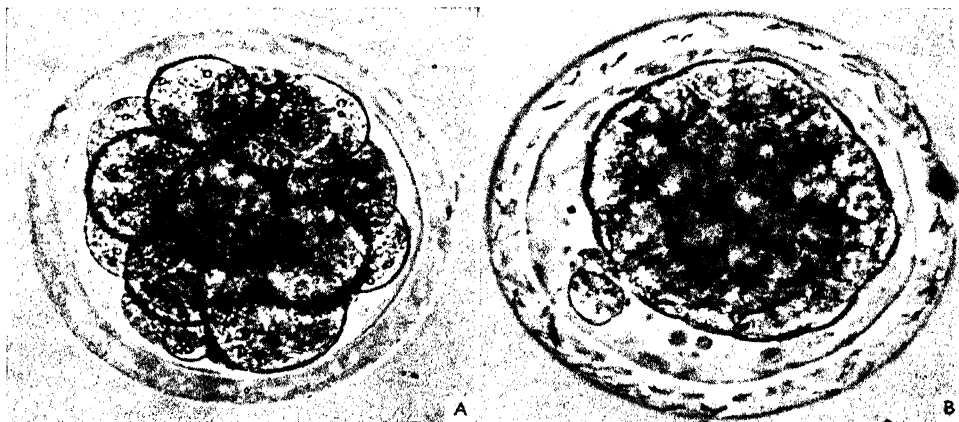


Fig. 14. 13—A. A living morula of sheep killed 5 days after mating. $\times 380$. B. A living early sheep blastocyst 6 days after mating. $\times 380$.

future embryo is located in one blastomere while that for the trophoblast is represented in the other. The occurrence of identical twinning, whatever its mechanism, does not support the idea of an absolute determination of blastomeric fates at this early stage. Further, most recent investigators have been unable to find the cytological differences between the first two blastomeres which van Beneden and the other embryologists cited in the above list believed to exist. For example, Heuser and Streeter (1929), in their careful work on cleavage in the pig, could find no demonstrable cytological difference between the first two blastomeres. They tentatively suggested, however, that these blastomeres were probably not identical and that, with the completion of the first cleavage, there has undoubtedly occurred some sorting of the constituent substances of the egg (*see, however, the experimental results of Nicholas and Hall, 1942, and p. 39*).



Fig. 14. 14.—Living blastocyst of mouse. $\times 480$.

In the second cleavage divisions of most mammalian eggs the larger blastomere usually divides first. Examples of this earlier division have been recorded by Lewis and Gregory (1929) in the rabbit, Heuser and Streeter (1929) in the pig, Lewis and Hartman (1933) in the monkey, and Amoroso *et al.* (1942) in the goat. Heuser and Streeter attach some importance to the difference in size and rate of division of the cells as affording the first evidence of sorting and localisation of special substances in the egg. They regard the more actively dividing cells as the precursors of the trophoblast while the more lethargic cells will give rise to the embryo proper. Hill and Tribe (1924) also believe that this separation occurs possibly as early as the second cleavage and

not later than the third. For the eggs of most investigated species, however, such a separation of cells for a definitive rôle in later development is not manifested at such an early stage of cleavage. There can be little doubt that, during cleavage, the blastomeres do separate into two groups—trophoblastic and formative—but the precise time at which the separation occurs varies in different species.

The morula in some species, e.g. the ferret, shows larger and smaller cells intermingled, whereas in others, e.g. the cat, the sheep, the goat and the pig, it possesses a distinct polarity with small actively dividing cells at the one pole and larger, less actively dividing cells at the other. Heuser and Streeter (1929) state that the smaller cells (presumed descendants of the larger blastomere) give origin to trophoblast, but that the larger cells are relatively undifferentiated and will give rise, as the result of further division, to yet more trophoblast and, after the last trophoblast cell has separated, to the inner cell mass. They further believe that the forces responsible for this differentiation are intrinsic and genetic rather than environmental.

Notwithstanding investigations on extensive and well-fixed material there is no conclusive evidence on the process whereby the trophoblastic cells come to enclose the formative inner cell mass. In *Hemicentetes* and *Elephantulus*, the latter is formed by migration into the interior from the blastocyst wall. For other

mammalian types van Beneden, in his work on the rabbit (1875), described a process of epiboly or overgrowth of the inner cell mass by the trophoblastic cells. Epiboly has also been recorded in the bat (Duval, 1895b; van Beneden, 1899) and in the cat (Hill and Tribe, 1924). Assheton (1898a) also supported it and in later papers (1908 and 1909) described the cells of the morula as separating into *epiblast* and *hypoblast*. He believed that the hypoblast cells came to surround the epiblast cells by a process of epiboly and that when the cleavage cavity appeared it separated the hypoblast into a layer—the future endoderm of current terminology—remaining against the deep (inner) aspect of the epiblast and a covering hypoblast—the trophoblast.

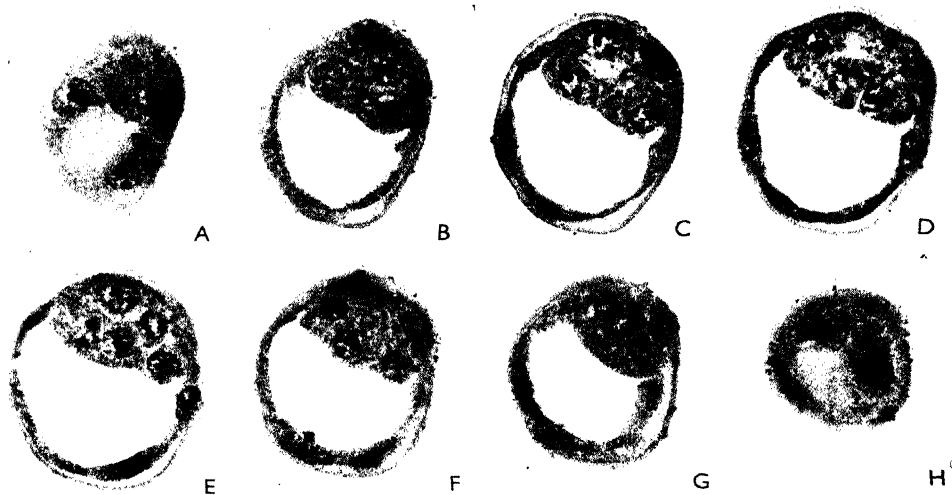


Fig. 14. 15 —Successive serial sections through a blastocyst of the golden hamster, *Cricetus auratus*. The trophoblast and inner cell mass are shown. $\times 520$.

Most recent work, however, suggests that the more slowly dividing formative cells give origin by radial cleavage to the smaller covering cells of the trophoblast. With the completion of the fifth and sixth cleavage, i.e. in morulae of 32 and 64 cells respectively, the outer cells of most mammalian eggs have acquired certain demonstrable and functional characteristics which mark them as the future trophoblast. With the functional differentiation of this trophoblastic layer, fluid begins to accumulate in the intercellular spaces between the trophoblast and the inner cell mass and converts these spaces into the blastocyst cavity. Heuser and Streeter have stressed the importance of this cavity as a necessary step for future implantation of the ovum. As a consequence of the method of formation of the blastocyst the inner cell mass is attached eccentrically to the inner aspect of the trophoblast. When the last trophoblast cell has separated from the inner cell mass the cells left inside the trophoblastic covering are the formative cells that give origin to those of the future embryonic disc (embryonic ectoderm and endoderm) and its derivatives, and, at least in some types, to the cells of the amnion.

The size to which the blastocyst grows subsequently is subject to considerable variation and is related to the type of subsequent implantation. It remains small (Figs. 14. 14 and 15) in many rodents (mouse, guinea-pig, hamster) and in the higher Primates (chimpanzee and man) where the implantation soon becomes interstitial (*see* p 80). It becomes large (Fig. 14. 22) in the types where implantation is central (e.g. ungulates, carnivores, lemurs and the macaque monkey).

The zygotes of viviparous mammals, with the exception of the opossum, normally reach the uterus during the cleavage stages, or as early blastocysts. In the sow (Heuser and Streeter, 1929) the egg reaches the uterus as a three-, four- or five-cell stage, 44–74 hours after mating; in the cat, on the other hand, the egg does not reach the uterus until the 7th or 8th day after mating (Manwell and Wickens, 1928). The tubal journey for most mammalian eggs takes from 3 to 3½ days (Hartman, 1939 and Hamilton and Laing, 1946) (*see also* p. 42 and Table III).

Mechanism of cleavage

It has long been realised that, even in micelithal types, the fertilised egg possesses an abnormally large amount of cytoplasm when compared with that of its nucleus and, *a fortiori*, of its chromosomes. R. Hertwig expressed the situation in terms of a “*Kernplasmarelation*.” He showed that a necessary consequence of cleavage, in which there is cell division without significant growth, is a rapid and marked increase in the numerical value of the ratio. That is, during cleavage there must occur either a segregation of nuclear material from the cytoplasm or a synthesis of such material from cytoplasm, deutoplasm or other sources. Since the early years of this century there have been numerous investigations, many of them with apparently conflicting results, on the nature and mechanism of this process, particularly with reference to the origin of chromosomal material (*see* Needham, 1931 and J. Brachet, 1944, for review). The work of the past ten years, however, has thrown much light on the chemical changes involved and it can be stated that there is an actual synthesis of desoxyribo- (thymo-) nucleic acid during cleavage. This synthesis is either from ribonucleic acid, which is presumably first reduced to its constituent nucleotides (Brachet’s “*synthèse partielle*”) or from purines, pentoses and organic phosphorus (Brachet’s “*synthèse totale*”). Hevesy *et al.* (1938), using “labelled” atoms, have shown that even organic

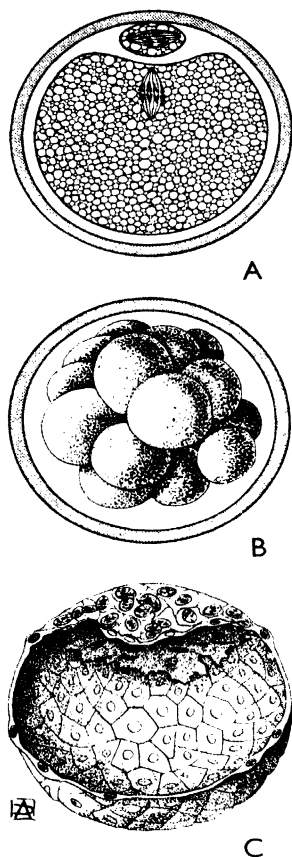


Fig. 14. 16—A. Schematic drawing of maturation division in the ovum of *Macacus rhesus*. B. Morula stage. C. A three-dimensional reconstruction based on serial photographs of a 9-day blastocyst. The pluripotential formative cells are shown as a lighter mass at the embryonic pole and are separated from the cavity of the blastocyst by a sheet of primary endoderm cells. *× 160*.

(Reproduced by the courtesy of Drs. Heuser and Streeter, and the Carnegie Institution of Washington.)

phosphorus can be involved in the synthesis. Much of the earlier confusion in the literature was due to absence of knowledge on the differences between desoxyribose and ribonucleic acids and of their respective rôles and distributions in the cell. The exploitation of the absorption spectrophotometer technique by Caspersen and of the Feulgen and other cytochemical techniques, including the use of nucleases, has clarified the situation though it must be stated that the basic chemical mechanisms are yet to be established. (For a detailed account of current knowledge *see* Chapter VI in J. Brachet, 1944.) Recently Thomas *et al.* (1946) have observed that the introduction of crystalline ribonuclease into the fertilised frog's egg inhibits or prevents cleavage. They interpret this result, however, as being due rather to an inhibitory effect on dehydrogenases than to depolymerisation of ribonucleic acid.

In part, at least, as a consequence of the abnormally low nuclear cytoplasmic ratio and of the extrusion of nuclear material in the polar bodies the mature unfertilised ovum is in a peculiar metabolic state. This is shown by many of its physiological characters, especially quantitative and qualitative changes in its respiratory mechanism, and a marked diminution in its permeability. At the time of fertilisation (or as the result of artificial stimulation, *see* p. 42) and accompanying the onset of the first cleavage division there are a number of alterations in the egg. These include an increase in permeability, modifications in the appearance of the cortical region with occasional disappearance of granules, possible elimination of osmotically active substances ("épuration" of Bataillon) and changes in respiration. Further, in eggs more intensively investigated, especially those of the sea-urchin, it is apparent that a number of hydrolytic processes become effective at this time. Thus there is a glycolysis, with acid production, proteolysis and liberation of ammonia, presumably at the expense of aminopurines. There are also changes in the electrolytes, notably a discharge of calcium. Brachet and others have suggested that this preliminary outburst of cytolytic-like activity (which Loeb actually considered cytolysis !) is followed in a few minutes by a partial synthesis of substances with an alteration in the physical, but not in the fundamental chemical, nature of the proteins. The process probably involves about 10 per cent. of the ovular proteins and can be regarded as a reversible denaturation. Rapkine (1931) pointed out, and has since produced much evidence in support of his suggestion, that such a partial denaturation could render SH radicles in proteins more accessible in the chemical activities of the cell (1933, 1938). As the sulphhydryl radicles are suspected of being concerned in stimulating cell division, and as the denaturation process changes the colloid state of the cytoplasm in the gel direction, the interest of the process is obviously considerable. Further, as Brachet suggests, it is possibly significant that all the activating processes in artificial parthenogenesis are capable of denaturing proteins (heat, acids, concentrated salt solutions, ultra-violet light, etc.).

Many investigators have studied the respiratory exchanges in cleaving ova. The results of this work, even when carried out by such refined and sensitive instruments as Linderstrøm-Lang's Cartesian diver ultra-microrespirometer, are difficult to interpret. There are marked differences in eggs of different species and at different times during cleavage but the respiratory changes do not appear, quantitatively, to be very important. Nevertheless there is a relationship between cleavage and oxygen consumption and the more rapid the former the greater the

latter. Some eggs, e.g. those of *Fundulus* (Loeb, 1894) and of the frog (Samassa, 1896, with numerous confirmations since) can cleave in the complete absence of oxygen, presumably by building up some variety of O_2 debt so that the cells must be regarded as facultative anaerobes. For details on changes in respiratory enzymes during cleavage the reader is again referred to J. Brachet (1944).

Monné (1944) believes that the position of the spindles in the course of the mitoses of cleavage is determined by the orientation of the fibrillae which constitute the structural phase of the cytoplasm (*see* p. 6). He further suggests that the speed of cleavage depends essentially on the relative proportions of enchylema and structural phase in the different cytoplasmic regions.

Rates of cleavage in mammals

The differences in the rates of cleavage of the eggs of different species have been commented upon by many investigators. The rate is inherent in the egg itself but can be influenced by environmental factors. Thus in amphibian eggs the cleavage rates of different species of *Amblystoma* at the same temperature are different, but in the eggs of single species the cleavage rate is to some extent a function of the temperature (Moore, 1939). Further, as Moore has shown, the range of temperature tolerance of different amphibian species varies from species to species.

The amount of stored deutoplasm (*see* p. 6) also markedly affects cleavage rates, e.g. the miclecithal egg of *Amphioxus* cleaves much more rapidly than the mediacithal egg of the frog, whilst the latter again divides more rapidly than the megalecithal avian egg. Amongst the eutherian mammals, although the temperature is relatively constant and there is little deutoplasm, differences in the rates have been recorded (Table III). It is possible to give only approximate data as relatively few observations have been made, and, in mammalian forms that discharge their eggs spontaneously, it is not always possible to "time" ovulation precisely. It can be stated, however, that rabbit ova cleave much more rapidly than those of any other type so far investigated. In small rodents such as the mouse, rat, guinea-pig and hamster, cleavage occurs at a slightly slower rate than in the rabbit, whereas the rate of cleavage in the larger ungulates is yet still slower.

That the differences in the rates of cleavage are inherent in the eggs has been shown by the studies of Castle and Gregory (1929) and Gregory and Castle (1931) on strains of rabbits which differ considerably in size. The cleavage rate is consistently more rapid in the larger-sized race than in the smaller-sized race. At corresponding ages the blastocyst stages from the former contain more cells than those from the latter. It was found, however, that although the eggs of the larger strain divide more rapidly, they differentiate at the same rate as those of the smaller strain.

Gregory and Castle point out that the higher developmental rate is influenced by the sperm as well as by the egg, and so it may be reasonably inferred that the rate is controlled by chromosomal genes. They suggest that a greater richness of the nucleus in such compounds as the sulphhydryl radicles (which Hammett, 1929, regards as the essential stimulus to growth by increase in cell number) may be responsible for the more rapid growth rate of the eggs and embryos of large race matings.

TABLE III

STAGES OF DEVELOPMENT, IN HOURS, OF DIFFERENT SPECIES

Type	Observer	Estimated time of ovulation	1-cell	Blastocyst	Time and stage at which egg reaches uterus	Total gestation period (days)
UNGULATA :						
Cow	Hamilton & Laing	10-15.5 hrs. post-oestrus	23-51.75	190	96 (8-16-cell)	284
Goat	Amoroso <i>et al.</i>	—	30.5	158	98 (10-13-cell)	150
Sheep	Clark	Late in oestrus	0-38.75	113.5-138.75	77-96 (16-cell)	150
Pig	Heuser & Streeter	—	0-51	114	44-74 (4-cell)	112
CARNIVORA :						
Ferret	Hamilton	31.5 hrs. after copulation	31.5-53.5	146-264	120-140 (up to 32-cell)	42
Cat	Manwell & Wickens	24 hrs. after copulation	—	—	190½ (blastocyst)	52-63
Cat	Dawson & Koters	29 hrs. after copulation	—	—	—	—
PRIMATES :						
<i>Macacus rhesus</i>	Lewis & Hartman	Determined by bimanual palpation	—	—	96 (16-cell)	164
RODENTIA :						
Mouse	Lewis & Wright	Any time during oestrus	0-24	74-82	72 (morula)	19-21
Rat	Gilchrist & Pincus	As early as 8.5 hrs. after copulation	8.5-27	—	—	22
Rat	Macdonald & Long	—	12-20 (15)	105-109 (107)	—	—
Rabbit	Gregory	10 hrs. after copulation	Up to 22	68-76	70 (blastocyst)	30-32
Guinea-pig	Squier	After copulation	3-30	115-140	80-85 (8-cell)	63

Later Gregory and Goss (1933) described a greater concentration of sulphydryl radicles as glutathione in the developing foetuses in a giant strain of rabbits and chickens than in the corresponding smaller size strains.

More recently Miller and Reimann (1940) have studied the effect of *dl*-methionine and of *L*-cysteine on the cleavage rate of rabbit ova *in vitro*. Their results indicate that in fact these thio-amino acids do stimulate blastomere proliferation. That no special environmental conditions affect the rate of cleavage in the rabbit is likely since the rate is similar *in vitro* and *in vivo*. On the other hand the eggs of mouse, rat, guinea-pig and hamster do not cleave in tissue-culture conditions. This may be due to some limiting factor which is absent in tissue-culture media, e.g. lack of some hormone or other stimulus. Burdick and Pincus (1935) have shown that ovarian hormones injected into the rabbit or mouse have no effect on cleavage. In tissue-culture experiments on cleaving rabbits' eggs, Pincus and Kirsch (1936) showed that the addition of oestrin in appreciable amounts does not affect the cleavage rate. The experiments appear to indicate that cleavage is not under hormonal control.

Physiology of cleaving eggs and blastocysts in mammals

Notwithstanding the extensive literature on the physiology of cleavage in invertebrates and lower vertebrates there has been very little work in the field on eutherian mammalian eggs. This is, in part, to be explained by the difficulty in obtaining eggs, especially in large numbers and of keeping them alive *in vitro*. It is also attributable to the fact that reliable ultramicro-methods have only recently been developed and have not yet been applied extensively to mammalian material. The earlier literature has been reviewed by Pincus (1936).

Apart from a few experiments by A. Brachet (1913) and by Long (1912) the pioneer work on the cultivation of mammalian ova *in vitro* was done by Lewis and Gregory (1929). These two workers showed, and their results were soon confirmed by Pincus (1930), that fertilised rabbit ova, isolated *in vitro* in homologous or heterologous sera and under ordinary conditions of tissue-culture, can cleave regularly up to the initial stages of blastocyst expansion, but collapse if cultured beyond this point. Ova grown in Carrel flasks or in the circulating cultures described by Pincus and Werthessen (1938) continue to expand after this stage. Such rabbit ova will only grow if cultured at a stage more than 10 hours after ovulation when they are still unicellular. Attempts at growing other mammalian eggs *in vitro* have not been so successful. Squier (1932) occasionally obtained division of the first blastomeres in the guinea-pig egg. Lewis and Hartman (1933) in *Macacus rhesus*, and Rock and Menkin (1944) in human ovarian ova have succeeded in culturing dividing eggs for a short period though in the latter case the divisions may be degenerative fragmentation. Other eggs, e.g. those of the mouse, rat and hamster are refractory to growth *in vitro*. The reasons for the special capacity of rabbit ova for growth and cleavage *in vitro* are not apparent, but it has been suggested that the albumen coat (Fig. 14. 11) may facilitate *in vitro* growth.

Most of the work on the factors involved in the normal cleavage of eggs has been done on invertebrates and lower vertebrates, and for surveys of the extensive literature on these types the reader is referred to Dalcq (1941), Child (1941), J. Brachet (1944) and Lehmann (1945). On the mammalian egg, however, there has

been important work by Nicholas and Hall on the potentialities of isolated blastomeres and a number of workers have studied the effects of the sex hormones and of certain chemical substances, notably colchicine, on mammalian cleavage.

As early as 1915 Huber suggested the possibility of growing isolated blastomeres of mammalian eggs and studying their separate development. Such experiments have been performed by Nicholas and Hall (1942). Utilising Hall's discovery that the zona can be dissolved in acid solutions (*see* p. 15) these workers found it possible to separate the first two blastomeres of rat's eggs and to introduce them into the uterine horn of a pregnant host female where pregnancy age, determined by an examination of the eggs, was either the same as, or slightly older than, that of the donor rat. When such transplantations were made over half the segmented blastomeres commenced development and caused mucosal reactions and decidua formation (*see* p. 63). Some of them formed egg cylinders and early embryos, but few attained later stages of development. When each of the blastomeres was transplanted approximately the same degree of development was attained by each. By fusing two single-celled eggs the workers obtained a single total oversized foetus. In this last experiment the foetus was shown to be of donor origin owing to its pigmentation, for the two fused eggs had been obtained from a pigmented stock. Nicholas and Hall conclude, from the results of these experiments, that "the rat egg possesses the capacity to satisfy two of the criteria for equi-potentiality, (1) each of the first two parts of the egg may form a whole embryo which develops further than the cleavage stages and, (2) the fusion of two eggs produces one single individual of large size." Further experimental work on these lines is needed and it will readily be understood how sterile it may render many of the conclusions based on the purely descriptive work on mammalian cleavage which has been considered earlier (p. 31). Nicholas (1947) has recently reviewed his experimental work on early mammalian development. The recent work of Meredith (1947) and of Fawcett *et al.* (1947) on the development of the ova of mice in the anterior chamber of the eye opens wide possibilities for further experimental investigation.

Experimental work, and especially *in vitro* culture of explants, on stages of mammalian development after blastocyst formation is more extensive and will be referred to later. A good review of their own work and of that of other investigators is given by Jolly and Lieure (1938). Once cleavage and blastocyst formation have been accomplished eggs that will not grow *in vitro* during the earlier stages are capable of considerable development under tissue-culture conditions.

Effects of chemical substances on cleavage

The importance of sulphydril radicals on the stimulation of cell division has been referred to earlier. Gregory and Goss (1933) showed that there is a higher concentration of SH groups as glutathione in the developing foetuses of giant strain rabbits and chickens than in corresponding smaller size strains. Further evidence for the importance of glutathione was provided by Pincus (1937) and by Pincus and Werthessen (1938). Miller and Reimann (1940) studied the effects of other thio-aminoacids (*dl*-methionine and *L*-cysteine) on cleavage in the rabbit eggs. With specified dosages of the tissue-culture media with one or other of these substances cleavage and growth of the ovum were definitely stimulated.

As has been stated earlier, progesterone, though apparently necessary for blastocyst expansion *in vivo*, has no obvious effect on cleavage in mammalian eggs. Tondüry (1943), however, who studied the effects of oestradiol and stilboestrol on the cleavage of eggs of *Triton*, obtained definite results. In concentrations of 1 : 300,000 to 1 : 500,000 of these substances he found that cleavage was markedly inhibited and that many abnormal mitotic figures were obtained ; on later stages of development the effects were variable but usually slight. Testosterone, however, was found to have little apparent effect on cleavage stages but influenced gastrulation and neurulation (Tondüry, 1941). These results, though enigmatic, are of considerable interest when it is remembered that the primary organiser may be a steroid.

Nebel and Ruttle (1938), Beams and Evans (1939) and Wilbur (1940) have all demonstrated that colchicine inhibits cleavage of the ova of *Arbacia*. Lehmann and Hadorn (1946) have made similar observations on the eggs of *Tubifex* and have contrasted the results with those obtained by treatment with benzoquinine. Keppel and Dawson (1939) and Samartino and Rugh (1946) have shown the same anti-mitotic effect of colchicine on the ova of frogs. A similar inhibition of cleavage of the eggs of rabbits *in vitro* was observed by Pincus and Waddington (1939). Waldo and Wimsatt (1945) have studied the action of colchicine on the cleavage of mouse ova *in vivo* and have recorded data on the nature and extent of the inhibition and on the fate of the affected ova. In the early cleavage stages the principal effects of the drug following subcutaneous or intraperitoneal injection were inhibition of cleavage evidenced by an increase in the incidence of ova having fewer than the expected number of cells, and irreversible damage as shown by an increased number of fragmented ova. Upon release from the inhibition some of the ova apparently resumed cleavage, but others underwent fragmentation. The production of living foetuses was significantly reduced in mice dosed with 1 : 8,000 colchicine intraperitoneally, or 1 : 4,000 subcutaneously. None of the mice receiving 1 : 4,000 colchicine intraperitoneally produced living foetuses. The increased inhibitory power of the intraperitoneal colchicine is apparently due to direct diffusion through the walls of the oviduct and not to its passage through the peritoneal opening in the ovarian bursa as has been suggested by Wimsatt and Waldo (1945). Kerr (1947) has extended the study of the effects of colchicine to later stages of development in the mouse and demonstrates that it causes embryonic disintegration.

Deutoplasmolysis (yolk elimination)

The phenomenon of the elimination of surplus yolk during maturation and fertilisation and during cleavage of the cytoplasm has been already referred to earlier (p. 26). It has been described for a number of mammalian eggs, especially in those containing a large amount of deutoplasm. Deutoplasmolysis was first described by O. van der Stricht (1909) in the ovum of the bat. The process occurs from the beginning of maturation, becoming accentuated during fertilisation and the early cleavage stages. A similar phenomenon was described independently by Hill (1910) for the egg of *Dasyurus*. In this marsupial egg the yolk is eliminated at the upper pole prior to, or simultaneously with, the formation of the first cleavage furrow. In the opossum McCrady (1938) has described the elimination not only of yolk but also of cytoplasm into the perivitelline space. This material

later comes to occupy the blastocoele and may be a source of nutriment. Deutoplasmolysis also occurs in the eggs of a number of other mammals—guinea-pig (Lams, 1913), *Didelphys* (Hill, 1918 and Hartman, 1919), cat (O. van der Stricht, 1923 and Hill and Tribe, 1924), pig (Heuser and Streeter, 1929), ferret (Hamilton, 1934), and horse (Hamilton and Day, 1945). In the egg of the horse, which is particularly rich in deutoplasm, a large amount of yolk is eliminated. Whether the cause of elimination is a phylogenetic one or a manifestation of dynamic changes in the ovum is not known. O. van der Stricht (1923) states that though the ovum of mammals is relatively poor in nutritive material, it still contains too much for the needs of the embryo, and so the surplus is expelled. It has been suggested by Levi (1915) that the elimination of the surplus material may be necessary in order to establish a normal nucleo-cytoplasmic ratio. It is also possible that the phenomenon represents Bataillon's "épuration" (see p. 35) or even that the eliminated material may have a hormone-like action.

Parthenogenesis in mammalia

Within recent years the question of parthenogenesis in mammals has been discussed by a number of investigators. Many observations have been made on the parthenogenetic development of invertebrates and lower vertebrates (Loeb, 1909 and 1913; and Delage and Goldsmith, 1913). The reader is referred to a review by Tyler for the literature up to 1941.

Pflüger (1863) commented upon the attempts to cleave in degenerating mammalian ova. Since that date many other investigators have contributed to the subject (see Bacsich and Wyburn, 1945b, for a brief review). The problem can be approached from two aspects—(1) parthenogenetic development of the unfertilised egg *in vivo*, and (2) activation of the unfertilised egg *in vitro*.

In the examination of some 500 unfertilised eggs of the opossum, recovered from the uteri of pseudo-pregnant animals, in which the environmental conditions were presumably suitable for development, Smith (1925) failed to find a single cleavage spindle in the eggs which were undergoing fragmentation.

Charlton (1917) working on the white mouse came to the conclusion that the cleavage processes had a superficial resemblance to parthenogenetic development but were really degenerative in nature. Lams (1926) on the other hand found that eggs may undergo several parthenogenetic divisions in the rat.

Krafka (1939) recorded a parthenogenetic egg in the human ovary and found parthenogenesis frequently in rodent ovaries. Bacsich and Wyburn, however, in their examination of an extensive series of guinea-pig, rabbit and rat ovaries, found that the condition is far from common. They did, however, find cleavage spindles, binucleated ova and a two-cell stage, also a "morula" in atretic follicles. The ova of the follicles undoubtedly have an inherent capacity to divide. Harman and Kirgis (1938) found that intra-ovarian ova that have passed through several cytoplasmic and nuclear divisions are found frequently in the guinea-pig, but no "far-going" parthenogenetic development of these ova was observed. See also Harrison (1948).

On the experimental side, the activation of mammalian ova has been carried out by Pincus (1936, 1939a and b) and by Pincus and Enzmann (1936). The latter found that a certain percentage of ova undergo a parthenogenetic cleavage when stimulated by a gradually developing hypertonic solution or supra-normal

temperature of the culture medium. Although all the material was not sectioned, cytological examination of some ova showed that these had segmented regularly and that the nuclei and cytoplasm were normal in appearance. It is claimed that viable parthenogenetic rabbits were secured as a result of these experiments. Pincus also found that foreign sperms may activate the egg to a limited degree, but only in certain instances do they penetrate the zona pellucida.

In the lower vertebrates it has been possible to stimulate the egg to develop by insemination with sperms treated in a number of ways which prevent the sperm nucleus from taking an effective part in development. This is known as gynogenesis. Gynogenesis can also be produced by fertilisation with foreign sperms, the chromatin of the sperm taking no part in the subsequent development. As Snell (1935), Amoroso and Parkes (1947) and others have shown, gynogenesis may occur to a limited degree in mammals as the result of irradiation of the semen. Eggs may be fertilised in the absence of the egg nucleus and undergo development, or partial development, solely under the influence of the sperm nucleus. This is known as androgenesis. Development of portions of an egg is also possible and is called merogenesis (see Needham, 1942, for review and discussion).

Twinning in mammals

In the human species, as is well known, two distinct types of twinning are recognised—*uniovular* (monozygotic) and *fraternal* (dizygotic). Triplets, quadruplets and higher degrees of multiple births are readily explained by polyovulation, by more than two centres of organisation in a single egg, or by combination of these two causes.

In mammals generally, however, as Hamlett and Wislocki (1934) have pointed out, there is much confusion in the use of the term "twins." Amongst the mammals three types can be recognised on the basis of the number of ova produced. There are :— (1) monovulatory species which usually discharge a single ovum at each ovulation ; (2) diovulatory species which normally ovulate two ova simultaneously ; and (3) polyovulatory species, in which more than two ova are discharged and in which the number varies from individual to individual and from one ovulation period to the next. Hamlett and Wislocki have suggested that the term "fraternal twins" should be restricted to species that usually bear only one or two young. Offspring of polyovulatory species, excepting the rare cases of polyembryonic origin, should be called *litter mates*. They also suggest that the terms "*synchorial twins*" and "*synchorial litter mates*" should be used for those instances where there has been a fusion of the membranes between fraternal twins or litter mates. The terms "*autonomous twins*" and "*autonomous litter mates*" can be used to designate cases in which fusion does not occur.

Passage of Mammalian Eggs down Oviduct, Free Blastocyst Stage in Uterus, Spacing and Migration

The mechanism whereby an egg, or eggs, after ovulation, can enter the uterine tube is still uncertain. In those instances where the ovary is surrounded by a closed, or practically closed, peritoneal capsule (ovarian bursa) the cilia lining the internal wall of the bursa together with possible fluid currents will ensure the entry of the egg or eggs into the abdominal ostium of the tube. In those species where

such a bursa is absent, as in women, it has been suggested that, at the time of ovulation, there is a kind of "erection" of the fimbriated extremity of the tube which results in the application of the infundibulum of the tube to the ovarian surface (Westman, 1937; Bovensiepen, 1942). There is, however, considerable evidence that eggs from one ovary can pass down the opposite uterine tube (transperitoneal or "external" migration) as was first demonstrated experimentally in rabbits by Leopold (1880) and since verified by other workers (Baur, 1921; Zweifel, 1922; Westman, 1926) and by the observations of Asdell (1924) and Hammond (1935). Bischoff (1842), however, by comparison of corpora lutea and distribution of embryos in rabbit does found no evidence of migration and Parkes (1924) found not a single conception in six rabbits in which one uterine tube and the contralateral ovary had been removed. In the rodents which possess an almost, or completely, closed ovarian bursa (e.g. mouse, rat, field vole) and in certain carnivores (e.g. the ferret) transperitoneal migration is extremely unlikely or impossible. In such rodents Hanson and Boone (1925) and Slonaker (1927) were unable to obtain any evidence for such migration with unilateral ovariectomy and contralateral removal of the uterine tube.

The mammalian uterine tube is lined with ciliated epithelium in which scattered non-ciliated cells can be found (Corner, 1932). At the time of ovulation the tubal musculature shows rhythmical contractions and the ovum is passed through the tube by these peristaltic-like contractions aided by the cilia. In most mammals the ova usually take three to four days to traverse the tube. In the dog and cat, however, the period is about seven days, but in the opossum it is about one day (Hartman, 1928; Smith, 1925). In general the time taken appears to be independent of the length of gestation and of the size, length and calibre of the tube. Thus, for example, eggs of the mouse and the pig take the same time to reach the uterus although the sow's uterine tube is about forty times longer than that of the mouse. For details on the tubal transport of eggs the reader is referred to Sobotta (1914), Westman (1926), Grosser (1927, 1942), Andersen (1927), Burdick *et al.* (1942), Grüner (1943), Courrier (1945).

The period during which the mammalian blastocyst is free in the uterine lumen varies from species to species and even, under certain physiological conditions, within a given species ("delayed implantation," *see later*). In the rabbit the free uterine period is about four days, implantation taking place on the seventh post-ovulatory day. The period in the rat, the mouse and the guinea-pig is normally rather less. The human blastocyst also implants on about the sixth post-ovulatory day (Hertig and Rock, 1945). In the macaque monkey implantation is later, not occurring until the ninth or tenth day after ovulation and in the cat it does not take place until the thirteenth or fourteenth day and is even later in the ungulates.

In some mammals the period between ovulation and implantation is either occasionally or regularly even longer. In these instances the time taken for passage down the uterine tube is about that normal for mammals but the greater ovulation-implantation interval is due to a longer free uterine existence on the part of the blastocysts. Such mammals are said to show *delayed implantation* (discontinuous development, developmental diapause).

There are two distinct varieties of delayed implantation: (1) physiological, and (2) natural. Physiological delay of implantation is found in a number of rodents (mice, rats, voles) and in certain insectivores (*Sorex araneus*, *S. minutus*).

In these mammals if a lactating female becomes pregnant the resulting gestation is distinctly longer than in the non-lactating pregnant female. This phenomenon was first described by Lataste (1887) and has since been verified and investigated by a number of workers; for reviews see Brambell (1937) and Courrier (1945). Notwithstanding the extensive literature, Brambell concludes that the explanation of the problem of how lactation brings about prolonged gestation is still to be discovered. He considers that the available evidence indicates an inhibitory effect of the blastocysts themselves, and exerted otherwise than through nutritional lack, rather than to an effect on the uterine mucosa. Since the blastocysts are free in the uterine lumen the inhibition must be exerted by way of a secretion into that lumen and Brambell tentatively suggests that it may be a growth-stimulating substance such as is found in certain embryonic tissue extracts.

"Natural" delayed implantation is now known, or strongly suspected, to occur in a number of diverse mammals—the roe deer (*Capreolus*), the polyembryonic armadillos (*Dasypus* (*Tatusia*) *novemcinctus* and *hybridus*), the European badger (*Meles meles*) and the American badger (*Taxidea americana*), and certain martens, weasels and bears. Hamlett (1935) has reviewed the problem and further details are given by Wright (1942), by Courrier (1945) and by Hansson (1947). Hamlett was inclined to attribute the phenomenon to a luteal insufficiency at the time when the blastocysts reach the uterus, but the data are scarce and inconclusive.

Spacing and Orientation of Ova in Mammals

In those mammals that have more than one young in a given pregnancy the embryos not only show a "spacing" along the longitudinal axis of the uterine horn but also exhibit a remarkably constant orientation within the lumen (Widakowitch, 1911; Mossman, 1937 and see p. 65). The spacing and orientation occur during that time when the egg lies free in the uterine cavity. During this time the blastocyst poles become apparent and its axis, relevant to the uterine axis, is determined. Even in the macaque monkey (Heuser and Streeter, 1941) and in man (Dickinson, 1933), where the uterus is simplex, there is evidence for some regulation of the position of implantation. Further, in some mammals the blastocysts implant in predetermined regions of the uterus, the remainder of the interior of which is apparently unsuitable.

The eggs themselves are distributed in the uterine lumen by irregular myometrial contractions and spacing can be explained in part by an absence of receptivity in the region of the uterine mucosa adjacent to an attached blastocyst (Mossman, 1937). The results, obtained by Fawcett *et al.* (1947), of transplanting several ova to the same eye lend some support to this suggestion. Mouse ova in the anterior ocular chamber appear capable of developing in close proximity only until the most "precious" one among them begins to implant when all but this implanting ovum degenerate. It appears unnecessary, therefore, to assume that the uterine mucosa participates in the creation of the local gradient of influence around the nidating ovum. Relative growth in later pregnancy "irons out" such minor variations in spacing as occur at the time of implantation. Markee (1944) studied the intrauterine distribution of sea urchin ova, and glass and celloidin pellets in the uterus of the rabbit. He found that the foreign bodies were distributed most evenly in oestrous does, nearly as evenly in ovulation groups, fairly evenly in does five days post-oestrus, but were not distributed in does ten days after

oestrus. He concluded that the ova were distributed by peristaltic contraction of the uterine muscle, and that such factors as contraction of the abdominal muscles, gravitational forces, ciliary action, intestinal peristalsis and body movement could be eliminated. In mammals with bicornuate uteri eggs can pass from

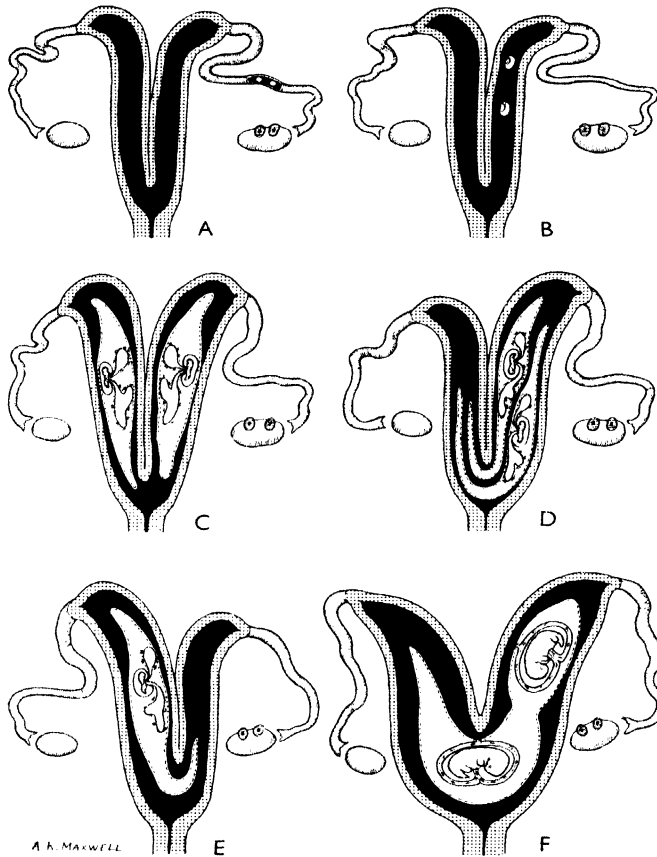


Fig. 14. 17—Schematic representations of the relationships between two ovulations from a single ovary and the distribution of the resulting conceptuses as found in a series of sheep. (From Boyd, Hamilton and Hammond Jun., 1944.)

one horn into the other as was first demonstrated by Bischoff (1845). This phenomenon (Fig. 14. 17), on which there is an extensive literature, has been reviewed by Boyd *et al.* (1944). It is known as trans-uterine (or "internal") migration and occurs in a large number of mammals (Table IV). Markee and Hinsey (1933) found that contractions of one cornu can drive ova into the other cornu, and Markee (1944) augments peristalsis with a recoil hypothesis and thus explains the movement of ova into the second cornu although their direction is opposite to that of the normal uterine peristalsis.

TABLE IV

TRANSUTERINE ("INTERNAL") MIGRATION IN MAMMALS

Type	Observer (names of observers who specifically describe migration are italicized)	Frequency	Remarks
"UNGULATA"			
Pig	<i>Corner</i>	50 % of cases	See footnote 1
	<i>Hausmann</i>	? 1 case in 17	—
	<i>Kelly</i>	—	Experimentally
	<i>Kupfer</i>	7 cases in 29	—
	<i>Warwick</i>	199 cases in 469	Also experimentally
Sheep	<i>Bonnet</i>	3 cases in 118	—
	<i>Curson</i>	2 cases in 5	Double pregnancies
	<i>Curson</i>	2 cases in 36	Single pregnancies
	<i>Hausmann</i>	1 case in 16	—
	<i>Present data</i>	7 cases in 17	—
Goat	<i>Quinlan</i>	1 case (triple pregnancy)	Quoted by Curson
	<i>Tsukaguchi</i>	1 case, at least, in 14	—
Cow	<i>Bergmann</i>	1 case in 50	See footnote 2
	<i>Day</i>	1 case in 1000	Personal communication
	<i>Kaltner</i>	0 cases in 300	—
	<i>Kupfer</i>	1 case in 1000	See footnote 2
Horse	<i>Day</i>	5 cases in 7	—
	<i>Kedrov</i>	—	See footnote 3
Deer (<i>Capreolus capreolus</i>)	<i>Bischoff</i>	—	—
<i>Procapra capensis</i>	<i>Turner</i>	—	—
	<i>Wislocki</i>	4 cases in 12	—
CARNIVORA			
Dog	<i>Bischoff</i>	Observed several times	—
	<i>Bonnet</i>	1 case in 12	—
	<i>Hausmann</i>	2 (? 3) cases in 17	—
	<i>Sparapani</i>	—	See footnote 4
	<i>Spiegelberg</i>	—	Quoted by Kussmaul (p. 319)
Cat	<i>Hill & Tribe</i>	1 case in several hundred	—
	<i>Markee & Hinsey</i>	—	—
Ferret	<i>Hammond & Marshall</i>	1 case	Experimentally

TABLE IV—*continued*

TRANSUTERINE ("INTERNAL") MIGRATION IN MAMMALS

Type	Observer (names of observers who specifically describe migration are italicized)	Frequency	Remarks
CARNIVORA—<i>contd.</i>			
Badger (<i>Meles meles</i>)	<i>Fischer</i>	—	Internal migration considered possible
Fox (<i>Vulpes vulpes</i>)	Personal observation	1 case	—
CHIROPTERA			
<i>Vespertilio murinus</i>	<i>Duval</i>	—	{ The blastocysts of these bats nearly always imbed in the right horn although there is ovulation from either ovary. Hence migration must occur.
<i>Vesperugo pipistrellus</i>	<i>Duval</i>	--	
<i>Rhinolophus ferrum equinum</i>	<i>Duval</i>	—	
<i>Molossus crassicaudatus</i>	Hamlett	--	
<i>Vesperugo murinus</i>	<i>Van der Stricht</i>	--	
<i>Myotis lucifugus</i>	Wimsatt	—	—
<i>Nyctalus noctula</i>	Personal observation	—	—
<i>Miniopterus dasythrix</i>	Matthews	—	—
(In <i>Glossophaga soricina</i> (Hamlett), where the uterus is simplex, and in <i>Cynopterus marginatus</i> (Keibel), where the uterine horns open separately into the vagina, migration has not been observed)			
INSECTIVORA			
<i>Blarina brevicauda</i>	Pearson	—	—
<i>Sorex araneus</i> Linn.	<i>Brambell</i>	28 cases in ? 99	—
<i>Sorex minutus</i> Linn.	<i>Brambell</i>	13 cases in ? 47	—
<i>Talpa europaea</i>	Personal observations	—	Not possible to correlate corpora lutea with pregnancies
<i>Erinaceus europaeus</i>	<i>Deanesly</i>	—	Not possible to correlate corpora lutea but embryos are fairly evenly distributed between the two horns
PRIMATES			
<i>Lemur rufipes</i>	Turner	1 case in 4	—
RODENTIA			
Rabbit	<i>Leopold Asdell</i> <i>Baur</i> <i>Brambell</i>	<i>External migration only</i>	{ Migration denied by <i>Bischoff</i> and ally, by <i>Parke</i> .

TABLE IV—*continued*

TRANSUTERINE ("INTERNAL") MIGRATION IN MAMMALS

Type	Observer (names of observers who specifically describe migration are italicized)	Frequency	Remarks
RODENTIA— <i>contd.</i>			
Guinea-pig	<i>Bischoff</i> <i>Leuckart</i>	— —	Migration denied by <i>Reichert, Squier, Kinney,</i> <i>Nicol and Kelly</i>
Albino rat	<i>Hanson &</i> <i>Boone</i> <i>Slonaker</i>	No evidence for migration No evidence for migration	— — —
Field vole (<i>Microtus agrestis</i>)	<i>Brambell</i>	No evidence for migration	—

Footnotes :

(1) In 1915 Corner considered external migration more probable ; in 1921 he produced evidence making internal migration a much more likely explanation.

(2) In the cow no records have been made of the migration in twins. Bergmann in two cases and Kupfer in seventeen, in none of which were both ovulations in the same ovary, failed to find migration. Lillie (1917 and 1923) gives no data on corpora lutea in his extensive study of free-martin twins in cattle.

(3) Kedrov, by rectal examination of 574 mares during 804 oestrous cycles, found that ovulation occurs 15-20 per cent. more often in the left ovary. As pregnancies were observed more frequently (25-30 per cent.) in the right uterine horn he concluded that intrauterine migration must occur.

(4) Quoted by Schmaltz (p. 233) as having found external (peritoneal) migration by experiments comparable to those of Leopold on the rabbit.

In the duplex uterus each uterus effects its own distribution and trans-uterine migration does not occur. The fair allocation of available space also occurs when a large number of ova are present (Krehbiel, 1941). The duplex type of uterus is capable of causing ova to migrate in a direction opposite to that of the uterine contractions and experiments by Lim and Chao (1926 and 1927) and Crabb (1934) have demonstrated that migration can occur in reversed uterine segments. Further, Nicholas (1939), who interposed a segment of intestine into a uterus, found migration into the lower uterine segment. These results strongly suggest that ova are distributed in an equitable fashion to the available endometrium irrespective of the type or the normal direction of contraction of the myometrium. They also suggest that trans-uterine migration might occur in a duplex uterus if the physical bar to such migration was removed. Negative results in attempts to achieve this in rats were obtained by Crabb (1934), but with more extensive experiments and improved techniques Krehbiel (1946) has obtained trans-uterine migration following a number of varieties of anastomoses between the two uteri.

On the question of orientation of the embryo Alden (1945) has shown that surgical reversal of the mesometrial—anti-mesometrial axis of the rat uterus causes no interference with the establishment of a normal embryonic axis. The embryos are correctly orientated, relative to the uterine axis, irrespective of the position of the altered segment of the cornu, and notwithstanding the altered gravitational relations.

VI. GASTRULATION

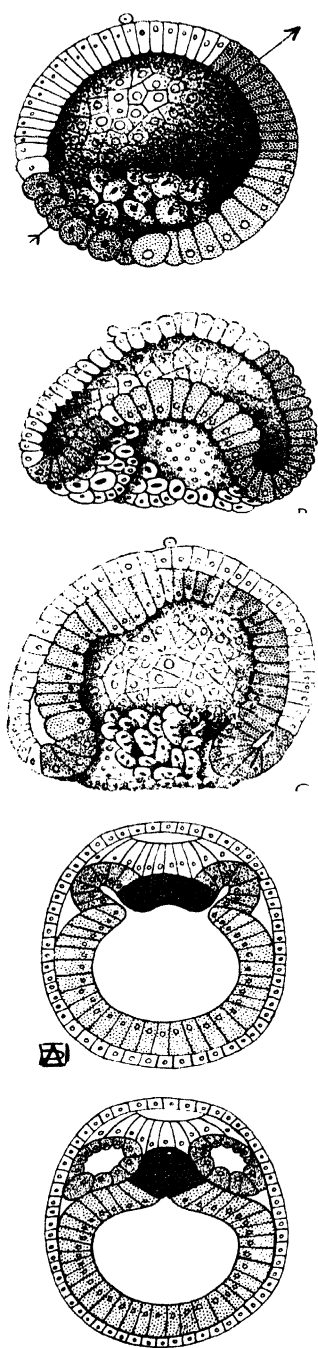
At the end of cleavage the metazoan embryo is in the *blastula* stage. During cleavage there has been some degree of segregation of the materials of the fertilised egg (see Nicholas, 1945, for details in *Amblystoma* and discussion). In the blastula stage, however, the embryo undergoes a rapid change in shape due to a complex rearrangement of its constituent cells. The process is called gastrulation, a term introduced by 19th-century embryologists to render explicit their phylogenetic beliefs in the significance of the rearrangement of the cells. It has as its result the establishment of the germ layers of the embryo. Modern experimental work and a more critical assessment of the relationships between ontogeny and phylogeny (de Beer, 1940) has caused the term gastrulation to lose its original significance. It is now regarded as essentially a phenomenon of cell movements that enable the presumptive organs to reach their definitive positions in the embryonic body (Rabl, 1915; Vogt, 1929; Dalcq, 1938, 1941; Pasteels, 1940; Lehmann, 1945), and not primarily a question of cell division and proliferation. The nature and extent of the movements, and their chronology, vary from species to species. In the mammals, gastrulation is delayed until the primary embryonic membranes have made their appearance, and is restricted to the cells of the embryonic disc.

For an adequate analysis of gastrulation it is first essential to know the position, in the late blastula stage, of the presumptive organ-forming areas. Satisfactory maps of these areas were first provided by Vogt for the amphibian egg by the application of coloured marks to the surface of different parts of the blastula and later following the changes in position of the marked areas during the processes of gastrulation. The use of dyes in the investigation of such problems had been attempted earlier by Goodale (1911a and b), Smith (1914, 1922) and Detwiler (1917), but it was Vogt's work (1925, 1929) that first demonstrated its efficacy in studying embryological problems. To quote Pasteels (1940) "son (i.e. Vogt's) mémoire fondamentale vint bouleverser de fond en comble nos notions sur la gastrulation des Vertébrés." Vogt's technique has been applied to the anamniote blastula by a number of other investigators (e.g. *Petromyzon*, Weissenberg, 1934, 1936; *Scyllium*, Vandelbroek, 1936; *Salmo iridaceus*, Pasteels, 1934; *Fundulus*, Oppenheimer, 1936).

The simplest type of gastrulation is one in which the embryo possesses only two germ layers—an outer *ectoderm* and an inner *endoderm*. Indeed, gastrulation is sometimes defined as that process whereby the unilaminar blastula is converted into a bilaminar gastrula.

Gastrulation in miolecithal eggs

The blastula that results from the cleavage of a miolecithal egg (e.g. that of *Amphioxus*) is a hollow sphere with the constituent cells of the vegetal hemisphere only slightly larger than those of the animal hemisphere (Fig. 14. 18A).



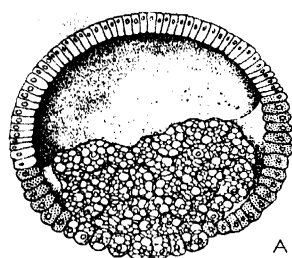
Gastrulation is apparently effected by a simple invagination of the vegetal hemisphere into the animal hemisphere (Fig. 14. 18B). The blastocoel is thus obliterated and a new cavity, the *gastrocoele* or *archenteron*, is formed. The invaginated vegetal pole cells lining the gastrocoele were originally believed to constitute the (*primary*) *endoderm* (Fig. 14. 18C) but as the result of the investigations of Conklin (1932) it is now realised that the inner layer includes presumptive mesodermal and chordal cells in addition to endoderm. Conklin's conclusions were based on cell lineage methods, but work on Prochordates (e.g. on *Ascidella*, Vandebroek, 1939) with more convincing techniques, lends strong support to his interpretation. The outer animal pole cells are the *ectoderm* and as yet uninvaginated mesodermal and chordal material. The opening of the gastrocoele, where the inner and outer layers are continuous, is the *blastopore*. Such a gastrula grows in length chiefly by cell proliferation at the blastoporic margin. This margin, therefore, was formerly considered to be an undifferentiated region and the cells arising there were considered to become ectodermal or endodermal depending on whether they migrated external or internal to the margin. It is now obvious, however, that the cells as the result of the localisation of presumptive material in the unsegmented egg, have a definite prospective significance prior to their migration. The morphogenetic movements concerned in bringing the mesoderm into the lining of the gastrocoele produce what was formerly called *secondary endoderm*. The primary, or true, endoderm becomes the cellular lining of the floor and approximately the lower two-thirds of the lateral walls of the primitive gut. The "secondary" endoderm, or chorda-mesoderm, consists of small yolk-free cells which form the roof and the upper third of the lateral walls of the primitive gut. In subsequent development they contribute little or nothing to the definitive gut. The medial axial strip

Fig. 14. 18—Semi-schematic drawings to show gastrulation and chorda-mesoderm formation in *Amphioxus*. (Based partly on Conklin, 1932.) Ectoderm and neural tube in section is unstippled. Endoderm is light stipple. Chorda mesoderm is dark stipple. Definitive chorda reverse stipple. Unsectioned mesodermal cells are shown with solid nuclei. A. Late blastula stage (longitudinal section). B. Early gastrula stage (longitudinal section). C. Late gastrula stage (longitudinal section). D. Early mesodermal diverticula stage (transverse section). E. Definitive coelomic pouch stage (transverse section). The arrow in A. represents the future antero-posterior axis of the embryo.

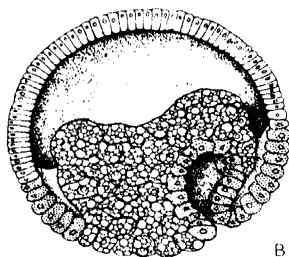
(chorda mesoderm), however, gives origin, by evagination, to the notochord. The lateral portions, as the result of segmental, paired, evaginations, become the mesodermal, coelomic pouches (Fig. 14. 18D and E). The first pair of these hollow coelomic pouches develop in the anterior or cephalic end of the developing embryo and the succeeding ones appear, in sequence, caudally towards the blastopore. The pouches increase in size, becoming interposed between the ectoderm and the primitive gut. Finally, by severance of their connection with the gut (primary endoderm) they form isolated paired mesodermal sacs along the length of the embryo. By yet further dorsal and ventral expansion of the sacs the endodermal gut is separated completely from the ectoderm except for dorsal and ventral mesenteries. The lateral wall of each coelomic pouch, in contact with the ectoderm, becomes transformed mainly into a segment of somatic muscle. The corresponding medial wall forms the splanchnic mesoderm of a segment of the gut. After the complete separation of chordal and mesodermal material the definitive gut is closed dorsally by apposition of the true endodermal cells. The processes just described obviously constitute the fundamental steps in the segregation and the differentiation of ectoderm, endoderm, mesoderm and the notochord in *Amphioxus*. Unfortunately, no significant experimental data are available for the egg of *Amphioxus*, but it can be presumed that the mechanisms of organiser activity and embryonic induction are similar to those found in the ascidian egg.

Gastrulation in medialecithal eggs

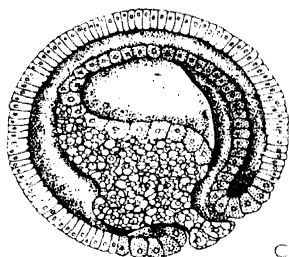
Gastrulation in the Amphibia, and especially in the frog (Fig. 14. 19), is the prototype for medialecithal eggs. With variations in detail, however, the process is very similar in the eggs of Polypteridae, Chondrostei, Amiidae, other medialecithal Amphibia and in the Petromyzontidae. In these types the greater size of the egg and the relatively slow cleavage rate of the yolk-laden blastomeres in the vegetal hemisphere are the main causes of the differences in gastrulation as compared with the process in *Amphioxus*. In Amphibia the blastula is spherical and distinctly polarised. The cells in the upper third (animal hemisphere) are small, usually pigmented, contain little yolk and are arranged in several layers to form the thin roof of the segmentation cavity or blastocoele. Those of the vegetal hemisphere are large, usually unpigmented and are heavily yolk-laden. These cells constitute too bulky a mass to be invaginated within the fewer and smaller cells of the animal pole. Consequently, gastrulation is effected by the proliferation and stretching of the latter downwards and over the vegetal hemisphere cells. Simultaneously, the animal pole cells of the advancing margin are progressively invaginated and a new cavity, the gastrocoele, which communicates with the exterior, is formed. Eventually the original segmentation cavity (blastocoele) is obliterated. The gastrocoele is lined in its ventral and lateral parts by (primary) endoderm, consisting of the original vegetal pole cells. In its dorsal and dorso-lateral parts, however, it is lined by animal pole cells (the equivalent of the "secondary" endoderm of *Amphioxus*). As the latter will give origin in subsequent development to mesoderm and notochord it is frequently called the invaginated chorda-mesoderm. The original experiments of Vogt (1929) and much work since (see Lehmann, 1945, for a review) have shown that this chorda-mesoderm is derived from a group of cells (presumptive chorda-



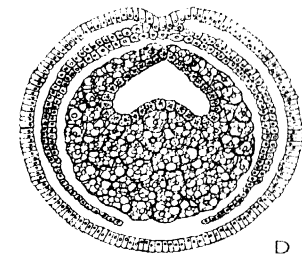
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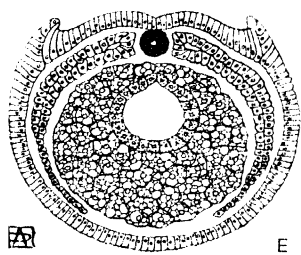
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mesoderm) occupying a specific topographical position on the surface of the pregastrulation blastula. This presumptive material can be traced backwards in development to a specific surface region in the un-cleaved amphibian egg. Those animal pole cells that are not invaginated during gastrulation and which were furthest from the initial point of invagination, become, in subsequent development, the surface ectoderm and the neural plate. The subsequent differentiation of these tissues is, to an important extent, dependent on the underlying chorda mesoderm (see p. 60, Organisers).

The actual migrations (*morphogenetic movements* or *Gestaltungsbewegungen*) of the cells in the process of amphibian gastrulation are complicated. Vogt analysed them into four major groups: (1) invagination; (2) dorsal convergence; (3) ventral divergence; and (4) epiboly. To these may be added a fifth—extension—though this belongs more to the post-gastrular stages. The details of these formative movements cannot be discussed here, but their integration and unity, in normal development, must be stressed. As Spemann (1938) has written, "One has throughout the impression that the single movements of all parts which constitute the general movement were all inseparably and causally connected with each other, somewhat like currents in a drop of water proceeding from one definite point." Bearing this unity in mind it is, perhaps, the more surprising that experiments demonstrate all the individual contributory processes to have been more or less determined at the commencement of gastrulation, for they take their course not only in collective action in the whole but also separately when isolated experimentally. There is a mosaic of formative tendencies or, as Vogt (1923) expressed it, the gastrula is "dynamically determined" in its constituent parts.

The process of spreading (epiboly) of the animal pole cells commences at the margin of the animal hemisphere and from the beginning is bilaterally symmetrical. Epiboly is more rapid at what will be the future cephalic margin (dorsal lip) of the blastopore and slower at the future caudal margin or *ventral lip*. The

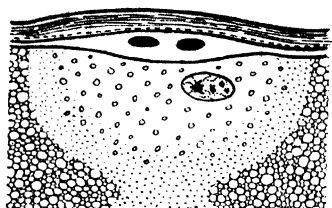
Fig. 14. 19—Semi-schematic drawings to show gastrulation, chorda mesoderm formation in Amphibia. The same conventions are adopted as in Fig. 14. 18. A. Early gastrula stage (longitudinal section). B. Gastrula stage (longitudinal section). C. Late gastrula stage (longitudinal section). D. Late gastrula stage through primitive streak (transverse section). E. Early neurula (transverse section). The notochord in E is represented by reverse stippling.

entire margin is the blastopore and, as epiboly nears completion, it becomes a small circular opening plugged with vegetal pole cells (yolk plug, Fig. 14. 19C). By this stage the original yolk-laden cells of the vegetal hemisphere of the blastula form a mass under the surface ectoderm and lying below the slit-like gastrocoele which is situated between the invaginated chorda-mesoderm (secondary endoderm) and the primary endoderm. The chorda-mesoderm overlaps the lateral margins of the primary endoderm (as a larger bowl could be inverted over a smaller one) and temporarily roofs over the gastrocoele. Soon, however, a median dorsal strip (the notochordal plate) of the chorda-mesoderm becomes evaginated to form the notochord just as occurs in *Amphioxus*. At the same time the edges of the (primary) endoderm extend medially and fuse ventral to the notochordal plate or, as it may now be called, the notochord. Mesodermal pouches are not formed in Amphibia. The bilateral mesodermal portions of the original chorda-mesoderm arise, by invagination, principally from the dorsal lip of the blastopore and extend between the endoderm and the overlying ectoderm. In the later stages of gastrulation the blastopore becomes constricted to form a dumb-bell shaped slit, the lower end of which remains open as the definitive anus. Its upper end remains open temporarily as the *neurenteric* canal which leads from the caudal end of the ectodermal neural groove into the gut cavity. Between these two openings the lateral lips of the blastopore become fused and the line of fusion is known as the *primitive streak*. From the primitive streak a mesodermal sheet continues to be proliferated on each side and spreads laterally and anteriorly, on each side of the notochord, as the embryo grows in length. Finally, the sheets meet ventrally and, as in *Amphioxus*, complete the separation of the ectoderm and the endoderm. In later development, as in other vertebrates, the sheet of each side forms mesodermal somites, nephrogenic cord and lateral plate mesoderm. In the last the coelom appears and separates an outer somatopleuric from an inner splanchnopleuric layer.

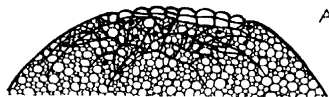
Gastrulation in megalecithal eggs

As is to be expected from the primary nature of the phenomenon gastrulation in megalecithal eggs varies with the amount of stored deutoplasm relative to the cytoplasm.

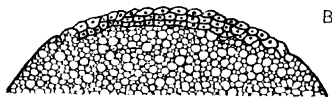
Pisces.—In the Teleostei the amount of cytoplasm in the egg is relatively large compared with that in the Sauropsida. At the end of cleavage in the teleosts the blastodisc or blastoderm covers about the upper one-fifth of the surface of the egg. At the margins of this blastodisc the more deeply situated cells separate, by a process of delamination, from the more superficial cells to form a circular zone of primary, or yolk, endoderm. Soon, however, an invagination of surface cells appears at the caudal margin of the blastodisc and forms a layer of "secondary" endoderm as in *Amphioxus* and the Amphibia. This layer spreads over the upper surface of the yolk and separates it from the overlying ectoderm. Around the periphery the "secondary" endoderm fuses with the yolk endoderm. A continuation of the epiboly results in the peripheral growing rim extending beyond the equator of the egg and constricting on the caudal aspect of the vegetal pole to form a circular opening, the blastopore. The two-layered blastoderm extends downwards to envelop the yolk mass and thus forms a bilaminar yolk sac which is part of the primitive gut and is characteristic of all megalecithal vertebrates.



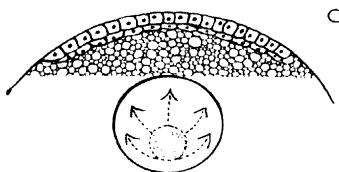
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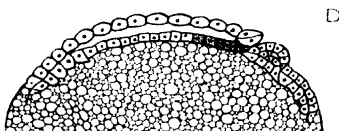
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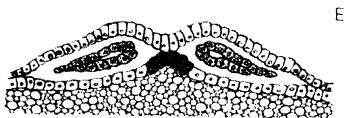
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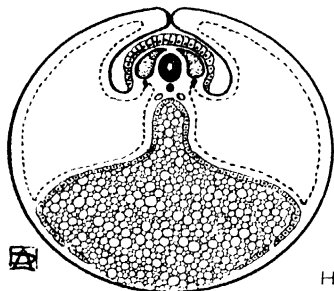
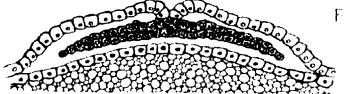
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Compared with gastrulation in the Amphibia the blastoporal invagination is much more marked and the primitive streak persists for a longer period. The notochord and the mesoderm arise in much the same fashion as in Amphibia, the former by invagination of chorda-mesoderm through the dorsal lip (region of primitive node) and the latter by invagination of ectoderm through the primitive streak (lateral lips of blastopore).

For further details on gastrulation in megalecithal piscine eggs the reader is referred to Pasteels (1936, 1940) and Oppenheimer (1936).

Reptilia.—In this vertebrate class the blastodisc is only a small area on the relatively large yolk mass and the cleavage cavity is insignificant. The cleavage cavity, in fact, is probably not homologous with that of lower types and should be called the sub-germinal cavity (Pasteels, 1945). It is generally believed that primary (yolk) endoderm cells separate from the surface ectoderm cells and become organised to form a complete sheet of primary endoderm. The bilaminar blastodisc then extends over the yolk, as in Teleosts. Coincidentally, a small depression appears on the surface of the disc near its caudal margin. This is an area of invagination and is considered to represent the dorsal lip of the blastopore. Ectodermal cells converge towards this area and a short primitive streak or "plate" is formed. There is, however, no movement of the actual edge of the bilaminar disc such as occurs in teleost development. From the primitive plate the notochord and mesoderm arise as in fishes and amphibia. For further details consult Peter (1934, 1935, 1937, 1938) and Pasteels (1937, 1940).

Aves.—The processes of cleavage and blastodisc formation in birds are very similar to those in reptiles. In the blastula stage the blastodisc forms

Fig. 14. 20—Semi-schematic drawings to show gastrulation, chorda mesoderm formation in the avian egg. A. Germinal disc with two polar bodies. B. Lateral surface view of early blastodisc. C. Longitudinal section through early blastodisc. D. Longitudinal section through later blastodisc. (The arrows in the inset show the direction of migration of the mesoderm.) E. Early gastrula stage (longitudinal section). F. Transverse section through late gastrula stage in region of notochordal plate. G. Transverse section at level of primitive streak. H. Section through somite stage to show formation of amniotic cavity and formation of yolk sac and extra-embryonic coelom.

a cap, three or four cells thick, on the top of the yolk and separated from the latter centrally by a shallow "segmentation" (cleavage or, probably best, sub-germinal) cavity but continuous with the yolk peripherally (Fig. 14. 20 B and C). The central part of the blastodisc, overlying the sub-germinal cavity, is called the *area pellucida*, the marginal zone, in contact with the underlying yolk, is the *area opaca*. There has been much discussion on the origin of the endoderm in birds (Patterson, 1909; Peter, 1938; Jacobson, 1938; Pasteels, 1945). The usual description, based largely on Patterson's work on the pigeon, but deriving from the earlier work of Duval (1884, 1888) is that yolk endoderm delaminates from the overlying ectoderm as in reptiles and the caudal margin of the blastodisc invaginates to form secondary endoderm in a manner similar to that found in the Teleostei. Convergence of the blastoporic lips gives origin to a distinct primitive streak and by their fusion the blastopore is closed. Jacobson (1938) has given an alternative explanation; he states that the "yolk endoderm" forms by delamination in the area opaca, but the "embryonic endoderm" arises at the posterior end of the area pellucida by the migration of single cells from a circumscribed region which he calls the primitive plate. This plate eventually bends inwards to form a typical archenteric canal, through which endoderm continues to be invaginated from the ectoderm.

More recently, Pasteels (1945) has reinvestigated the problem of the origin of the avian endoderm. He considers that, in the duck, the primary endoderm is the result of a progressive delamination of the cleaving blastodisc which separates the superficial cells from the deeper ones. In this conclusion he has verified earlier statements by Peter (1938), who stated "Das innere Keimblatt entsteht also nicht durch Einwandern von Zellen sondern *in loco* durch Abspaltung von einer einheitlicher undifferenzierten Zellmasse." Unlike Peter, however, who considered that such a formation of a bilaminar embryo must be excluded from gastrulation, Pasteels homologises this stage of avian development with the amphibian blastula. He considers, therefore, that the cleft separating the ectoderm and the endoderm is the homologue of the blastocoele and that the Sauropsidan "subgerminal cavity" is quite different, being, in fact, "only the result of a progressive liquefaction of the yolk acted upon by enzymes produced by the yolk syncytium and perhaps by the blastoderm itself." (Cf. p. 23.) Pasteels has advanced cogent arguments in support of his conclusions and, while it is not yet possible to make the positive assertion that his explanation is the correct one it must be admitted that it fits in better with our knowledge of gastrulation in other vertebrate types than the available alternative explanations.

In later avian development, material of the primitive streak (plate) invaginates between the ectoderm and the primary endoderm. On Pasteels' interpretation this process is equivalent to that found in the Amphibia and the invaginated material gives origin to the chorda-mesoderm more or less as in lower vertebrates.

Mammalia.—There is an extensive and conflicting literature on gastrulation in mammals due, partly, to the absence of suitable experimental techniques for the investigation of the problem. Keibel (1889) and Hubrecht (1890) considered that it occurred in two stages: (1) production of primary endoderm by delamination and (2) invagination resulting in the production of "secondary" endoderm, mesoderm and notochord. In a later paper, however, Hubrecht (1908) concluded that the production of primary endoderm should alone be regarded as gastrulation. Rabl (1915) and most subsequent workers in this field regard invagination as an

essential part of mammalian gastrulation, though it is much modified and reduced as compared with anamniotes. Comparative embryological investigation and the little experimental work that has been done on early mammalian development (cf. Waddington, 1940) strongly support such a conclusion and this interpretation is accepted in the following discussion.

The early development of the monotreme egg has marked resemblances to that of the eggs of reptiles and birds, but, according to Flynn and Hill (1942 and 1947), the origin of the endoderm in monotremes is similar to that in marsupials. These workers have shown that the monotreme blastoderm, at a very early stage of development, possesses a nucleated syncytial germ-wall, similar to that in reptiles and encircling the blastodisc proper. This germ-wall is formed by the fusion of cells (vitellocytes) derived from the peripheral cells of the blastoderm as early as the 41-celled blastodisc. The germ-wall plays a very important rôle in development though it makes no cellular contribution to the blastodisc. It is endowed with the power of migration and moves out over the yolk as a continuous ring, and this peripheral migration conditions the increase in surface-extent of the blastodisc and its concomitant conversion from a thick biconvex disc (six to seven cells in maximum thickness) into a thin blastoderm (two to three cells in maximum thickness). In the cells of the blastoderm two types can be distinguished: (1) prospective ectodermal cells which are larger, more numerous and more lightly staining, and (2) primitive endodermal cells which are smaller, less numerous and more deeply staining. According to Flynn and Hill these two cell varieties show no regular arrangement and occur intermingled with each other throughout the thickness of the blastoderm. Finally, the blastoderm becomes unilaminar, as was first observed by Semon (1894) and in it the two types of cells, prospective ectodermal and primitive endodermal, can still be recognised. According to Flynn and Hill the "attainment of this unilaminar condition heralds the commencing formation of the primary germ layers. Its establishment ensures that all the prospective ectodermal cells have reached their definitive position at the surface and it only remains for the primitive endodermal cells to sort themselves out and acquire a deep position below them." It is largely by migration that the endodermal cells reach their final deep position beneath what becomes a continuous layer of flattened ectoderm. Finally, the endodermal cells unite to form a continuous layer. The two primary germ layers in monotremes, therefore, are formed by the segregation of the two types of cells in the unilaminar, and earlier, blastoderms, into two distinct layers, a superficial ectoderm and a deep endoderm.

As Flynn and Hill have stressed, this method of formation of the ectoderm and the endoderm has remarkable similarities to the method of primary germ layer formation in marsupials. In *Dasyurus* (Hill, 1910), for example, the unilaminar blastocyst consists of "formative" and "non-formative" portions. The latter gives origin to the trophoblast and possesses only one variety of cell. The formative region, however, is composed of prospective ectodermal and primitive endodermal cells which possess not only the same potencies but also much the same cytological characters as those of the monotreme. Similar findings have been recorded by Selenka (1886) and Hartman (1919) for the origin of the endodermal cells in *Didelphys*, though these workers did not find the cytological distinctions in the unilaminar stage, and, in particular, Hartman could find no polarity in the blastocyst. McCrady (1938, 1944) indeed, who also studied *Didelphys*, suggests

that "the originally unilaminar blastocyst is at least as homogenous as the amphibian blastula and probably quite as plastic." He believes that the first step in visible differentiation is the migration of endoderm mother cells (Selenka's "Urenodermzellen") into the interior of the blastocyst. Some of the cells are probably part of the axial mesoderm for, in later stages, as the prochordal plate, they proliferate cephalic mesoderm. McCrady considers it probable that it is this prochordal plate material in the endoderm which induces the formation of a neural (medullary) plate represented, at first, by only the brain plate portion.

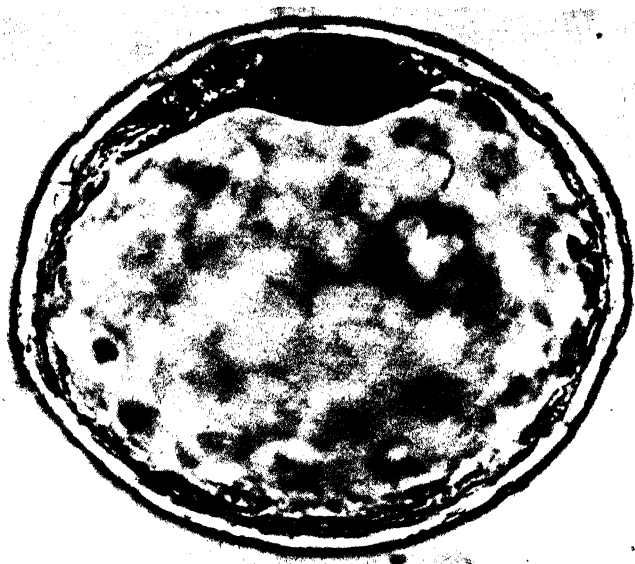


Fig. 14. 21 Living sheep blastocyst 8-days post insemination.
X 320.

In the eutherian mammals two species—*Hemicentetes* (Goetz, 1938) and *Elephantulus* (van der Horst, 1942)—possess a "mammalian blastula" similar to that of marsupials, and in them the endoderm arises by migration of cells inwards from the unilaminar blastocyst wall. In other eutherian blastocysts the endoderm arises by migration of certain cells from the inner cell mass, or by "delamination," to form a layer of endoderm which if, as is usual, the extra-embryonic mesoderm is not yet present, spreads round the interior of the blastocyst to form a bilaminar omphalopleure (see p. 93 and Figs. 14. 16, 21 and 22). In certain mammals, however (e.g. man), extra-embryonic mesoderm appears precociously and the endoderm from the inner cell mass forms a small primary yolk sac which has no direct contact with the trophoblast.

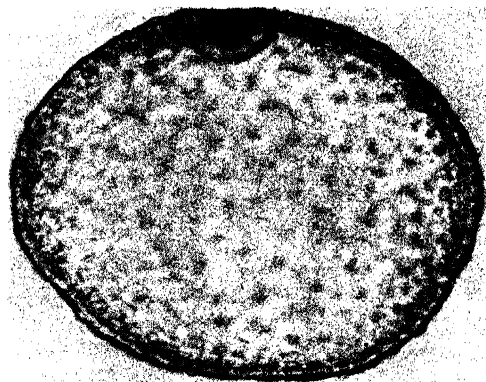
From the above descriptions (which can be amplified by reference to Hill and Tribe, cat, 1924; Streeter, 1927, and Heuser and Streeter, pig, 1929; Hamilton, ferret, 1937; Heuser and Streeter, *Macacus rhesus*, 1941, Hamilton *et al.* 1945) it will be seen that endoderm formation, as a stage in gastrulation, is essentially similar to that in the avian egg, i.e. cells migrate from the blastoderm and come to be on

its lower surface, where they form a layer of endoderm. Whether this process is by "delamination" or by a diffuse migration (poly-invagination, *see* Patterson, 1913, *Dasypus novemcinctus*) appears to vary from species to species and to have no obvious significance. Further there appear to be non-significant species differences in the precise period at which cytological characteristics are established in the primitive endoderm cells and in the exact area from which the migration occurs. The origin of the endoderm in mammals, with the exception of the monotremes, is of special interest in that this germ layer forms a yolk sac although the eggs are miclecithal. Such a yolk sac has always been considered strong evidence in support of the generally accepted theory that the ancestors of mammals possessed

megalecithal eggs. Notwithstanding the relationship of part of this primary endoderm to an extra-embryonic membrane there can be no reasonable doubt that the mammalian method of primary endoderm formation must be considered an integral part of gastrulation.

Although there is no experimental work on the morphogenetic movements in the mammalian primitive streak region the numerous descriptive accounts available for the early mammalian embryonic disc justify the assumption that there is a second phase in the process of gastrulation.

This is by invagination of notochord and mesoderm in much the same manner as in reptiles and birds. The chorda mesoderm is temporarily



14. 22—Living sheep blastocyst 10-days post insemination, with endoderm cells visible on the deep surface of the inner cell mass. $\times 106$.

intercalated in the roof of the archenteric cavity. Thus in human development the intra-embryonic mesoderm arises for the most part from the ectoderm in the region of the primitive streak. This, however, is not solely concerned with mesoderm formation, for in the monkey (Heuser and Streeter, 1941) and in other mammals the cells in the primitive streak region are pluripotent and are also capable of giving origin to further ectoderm and even to endoderm (Fig. 14. 23). In human embryos of about 15 days' gestation age (e.g. the Edwards-Jones-Brewer embryo, Brewer, 1938) the primitive streak appears as a limited proliferation of the ectoderm at the posterior edge of the embryonic disc. The disc grows mainly by the proliferation of cells in its caudal region. Hence the primitive streak becomes elongated by the addition of new ectodermal cells at its caudal end and the oldest part of the streak is situated cephalically. The intra-embryonic mesodermal cells that arise from the primitive streak pass laterally, and forwards, between the ectoderm and the endoderm and eventually come into contact with the extra-embryonic mesoderm covering the amnion and yolk sac. The anterior extremity of the primitive streak shows a special ectodermal proliferation, known as *Hensen's node* or the *primitive knot*. An invagination of the ectoderm occurs in the centre of Hensen's node. This invagination is the blastopore and it

possesses dorsal (or anterior), ventral (or posterior) and two lateral lips; the ventral is continuous with the primitive streak. From Hensen's node a cord of cells passes forwards beneath the ectoderm and in the middle line. This cord is the *notochordal* or head *process*. The blastoporal invagination then extends forwards into the notochordal process to form the *notochordal*, or *archenteric*, canal. Since the anterior end of the notochordal process is relatively fixed further growth in its length is brought about mainly by a proliferation of cells at Hensen's node which, with the primitive streak, migrates in a caudal direction as the notochordal process and the embryonic disc increase in length. Meanwhile the notochordal process has fused with and become intercalated into the primary endoderm. Openings appear in the floor of the notochordal canal and thus a communication is established between the yolk sac and the amniotic cavities. Later the openings in the floor of the notochordal canal become confluent and form a deep longitudinal groove on the under-surface of the notochordal process. The latter then increases in breadth and



Fig. 14. 23 —Section through Hensen's node of a 14-day post insemination sheep embryo. The yolk-sac is now completely formed and mesodermal cells are interposed between the embryonic ectoderm and endoderm. The extra-embryonic coelom can be recognised at each side. ... 100.

becomes flattened to form a plate of columnar cells, the *notochordal plate*. This differentiation commences cephalically and extends in a caudal direction as the notochordal process elongates in association with the proliferation and backward migration of Hensen's node. It is thus that notochordal tissue becomes temporarily intercalated in the endodermal roof of the yolk sac. Later, a modified folding process begins in the anterior part of the notochordal plate, and by gradual extension caudally, separates the notochordal tissue from the endoderm. The latter then fuses in the dorsal mid-line to complete the definitive endodermal lining of the yolk-sac.

It will be seen from this description that, considering the differences in egg structure and the fact that the mammalian egg forms membranes quite unknown in amphibian development there are striking resemblances in gastrulation in these two vertebrate classes.

Significance of gastrulation

As has been indicated earlier, gastrulation, from a descriptive point of view, has as its main result the establishment of the three germ layers and the bringing of

the principal organ primordia into their proper positions in the embryo. The morphogenetic movements in embryo formation and what Child (1941) has called the "formal regional pattern" are strikingly similar in all the vertebrate classes. Even in the development of the mammals the gastrulation changes, though complicated by certain features associated with intra-uterine environment, do not differ fundamentally from those found in other vertebrates. Further, the general patterns of embryo formation in prochordates (*Amphioxus* and the ascidians) show resemblances to that of vertebrates. The "visible" results are brought about, in the first instance, mainly by extensive cell movements though, later, proliferation plays an important rôle. The experimental study of embryology in the present century, however, has shown that other profound "invisible" changes occur during gastrulation. Thus transplantation, explantation and other experiments have demonstrated that during, or shortly after, gastrulation some of the principal organ and tissue primordia become more or less rigidly "determined," i.e. their fate is definitely fixed.

The concept of *determination* is fundamental in modern embryology. Before the developmental fate of a part of an embryo has been fully established, i.e. while it is in the *pluripotent* or *plastic* state, it is said to be undetermined. In normal development the actual fate ("prospective significance" of Driesch) of a region is that chosen from amongst the possible fates ("prospective potency" of Driesch) and one of the most fundamental ontogenetic processes is the progressive restriction, or determination, of the possible fates of the different parts of the egg and blastula as development proceeds. Many investigations, principally those of Spemann and his school (summarised in 1938), have shown that up to a certain stage in gastrulation the fates of most of the embryonic areas, except that of the blastoporic region, are undetermined. Thus if a portion of one "presumptive" region is grafted into another region of different presumptive fate it will develop in conformity with the developmental tendency of the receptor region (dependent differentiation, *Ortsgemäss* of German embryologists, "neighbourwise," Needham, 1942). During gastrulation this pluripotency becomes restricted so that the main fates of the different embryonic regions are irrevocably determined, and a transplanted portion grafted after this stage develops in accordance with its own prospective significance (self-differentiation, *Herkunftgemäss* or "selfwise"). As Needham (1942) has expressed it, during gastrulation "prospective potency has been ruthlessly curtailed to prospective significance."

Organisers

One region of the amphibian embryo was shown by Spemann and Mangold (1924) to be determined at a very early stage, possibly at the time of fertilisation and it is, therefore, much less plastic than the other regions of the fertilised egg. This is the region of the dorsal lip of the blastopore which, as has been seen, gives origin, in the later stages of gastrulation to the notochord and the intra-embryonic mesoderm. The dorsal lip region develops "selfwise," and in no direction other than its normal presumptive fate. If all, or part of it, is grafted into the undetermined tissues of another blastula or early gastrula, the host cells in its neighbourhood irrespective of their presumptive fates, are caused to form or to attempt to form, the tissues of a secondary embryo. Further, if the dorsal lip region is absent from an early embryo normal development does not take place. The formative

influence exerted by the dorsal lip region on neighbouring regions is called *induction*. As the dorsal lip cells appear to contain within themselves the ability to determine the fates of other regions, Spemann called the region in which they are found the *primary organiser* as it is apparently the first of such regions to function in development. Further analysis of this organiser has shown that, in lower vertebrates, it can be divided into a *head organiser* (consisting of the first cells to be invaginated —prochordal plate) and a *trunk or tail organiser* (constituted by the later invaginated chorda-mesoderm). The conception of organiser activity has been applied to many other stages of development and secondary (second grade), tertiary (third grade), etc., organisers are now recognised as being concerned with the initiation and control of many, if not all, of the processes of early development. Individual organisers do not usually retain their inductive ability for more than a limited period during which the cells or tissues upon which they produce their effects are said to be *competent* to react to their influence (Waddington, 1932). The inductive effect of an organiser results normally in the production not only of a particular tissue (e.g. neural tissue or lens of eye), but also in the determination of the regional character of the resulting tissue (e.g. if neural tissue is concerned, midbrain or spinal cord). That is, induction results in two kinds of determination (Needham *et al.*, 1934) :— (1) the presence of a particular tissue (*evocation*), and, (2) the character which that tissue adopts (*individuation*).

The concept of the primary organiser and of the organiser region has possibly been pushed too far by some investigators. Indeed for some, as Lehmann (1945) has put it, the concept has taken on “ eine beinahe mystische Verwachsenheit ! ” It is impossible to go fully into the problem here and the reader is referred to Spemann (1938), Weiss (1939), Waddington (1940), Daley (1941), Child (1941), Needham (1942), Brachet (1944), Tondury (1941 and 1942), Lehmann (1945), Woerdeman and Raven (1946), for further details and for discussions on the nature of organiser activity. For recent work on the nature of the movements of embryonic cells see Holtfreter 1947a and b.

Significance of the germ layers

The concept of three primary germ layers has been very important in the history of embryology. The results of modern experimental embryology have, however, rendered untenable too rigid an adherence to a concept of this absolute specificity. The germ layers have a topographical significance and their formation appears to be “ the embryo's method of sorting out its constituent parts ” (Oppenheimer, 1940) for, like the blastomeres, the germ layers have a prospective significance and a prospective potency. The former is what they actually become in normal development, the latter is what they are capable of becoming under diverse natural or experimental influences. As McCrady (1944) has emphasised, however, much of the difficulty can be avoided by being careful not to use the names of any of the germ layers before differentiation has taken place ; in other words to make a clear distinction between germ layers and prospective germ layers. McCrady goes even further and writes “ whether there is a labile or plastic stage in the early precursors of the germ-layers or not, there is always a mosaic stage in which the germ-layers are rigidly determined and non-interchangeable. And this mosaic stage is the basis of the significance of the germ-layers in the physiology of development.”

VII. PRE-IMPLANTATION CHANGES IN THE UTERUS

The histological changes that occur in the endometrium during the oestrous cycle have been described in detail in many animals. These changes are under the control of the ovarian hormones (Chap. 3) and thus related to the activities of the hypophysis.

The changes that occur preceding and succeeding ovulation prepare the endometrium for the reception of a fertilised ovum or ova. In the absence of fertilisation the endometrium undergoes regressive changes. In some animals, e.g. the pig (Corner, 1921), these changes affect mainly the epithelium of the endometrium. In the higher primates, including man, there is an extensive endometrial proliferation before and after ovulation. In the absence of fertilisation this endometrium undergoes degeneration at the end of the luteal phase, so that most of it is shed at the next menstrual period.

Nutritive material, in the form of glycogen, lipides and other substances, accumulates in the epithelium and stroma of the endometrium, and may provide nourishment for the embryo in the event of pregnancy (*see* Alden, 1947, for recent literature). The amount of these substances varies not only in different species but also shows a cyclical variation in the individual females of a single species. The differences, however, do not appear to be fundamental, and are probably only adaptations to the particular problems involved in the different methods of implantation.

There is general agreement that the presence of the luteal hormone is essential for the successful implantation of the blastocyst, but the mechanism through which it makes implantation possible is not known (*see* Ancel and Bouin, 1910 and 1924; Corner and Allen, 1929; Courrier, 1935; Courrier and Kehl, 1938; Pincus and Werthessen, 1938; and Phelps, 1946). Corner (1928) found in rabbits that progesterone is necessary not only for implantation but also for the nutrition of the blastocysts during the three or four days they are free in the uterus. In the mouse, Bloch (1939) has described the secretion of an osmophilic substance by the uterine epithelium which, she believes, is absorbed by the free blastocysts. The work of Daron (1936), Markee (1940), and, particularly, of Phelps (1946) has demonstrated that there is an increased blood supply just below the uterine epithelium at the time the blastocyst is about to implant. This ensures an adequate supply of nutritive material to the endometrium. It is possible that some chemical or hormonal substance is produced by the fertilised ovum which is responsible for the continued activity of the corpus luteum. Bac-sich and Wyburn (1940, 1941, 1942a and b and 1944) have studied the cyclical endometrial vascular changes in the uterus of the guinea-pig. At oestrus they found that there is relative antimesometrial hyperaemia. This, they thought, might in part determine the antimesometrial implantation of the blastocyst.

During the progestational period there is a decrease in the muscular activity and tonicity of the uterus and this facilitates the retention of the ova in the uterus.

There are two contrasting views held regarding the respective rôles of the trophoblast and the endometrium in the process of implantation. One group of investigators has considered that the trophoblast is the active agent while the maternal tissue remains passive, e.g. Spee (1901) in the guinea-pig blastocyst described pseudopodia-like projections of the implantation pole penetrating the

epithelium, and Assheton (1894) and Schoenfeld (1903) explained implantation as the effect of the expansion of the blastocyst on the uterine epithelium. This view has support by Brambell (1935), and Brambell and Perry (1946). Wimsatt (1944), in describing implantation in the bat, came to the conclusion that the changes in the epithelium of the "pocket" into which the blastocyst comes to rest is "an expression of a localised physiological reaction of the uterus to some chemical stimulus of unknown nature liberated by the ovum, which may produce this effect either by acting locally on the epithelium or by inducing a local relaxation in the uterine musculature." The view that the egg is passive and the uterine wall is active has support from Huber (1915) and Kirkham (1916). The formation of deciduomata in some species, by mechanical stimulation of the sensitised mucosa, shows that the uterine epithelium may react in the absence of a blastocyst (*see* Loeb, 1908 and 1909; Selye and McKeown, 1935, and Hisaw, 1935). Krehbiel (1937) and Mossman (1937) demonstrated that an embryo is not essential for the development of the decidual reaction, and that the stimulus need not be specific. Thus the formation of deciduomata can be induced experimentally by mechanical or electrical uterine stimulation in pregnancy, pseudopregnancy, lactation and certain other conditions. Such induced "maternal placentae" are extremely similar to the decidua of pregnancy both in histological structure and vascular pattern and the surface epithelium of such deciduomata may be shed with resulting haemorrhage into the uterine cavity (Selye and McKeown, 1935; Krehbiel, 1935). It has, indeed, been suggested that such extravasation of blood is a normal feature of decidual development and does not depend on the trophoblast. Atkinson (1944) has shown that deciduomata in the pseudo-pregnant mouse have an inherent "life span," provided that an adequate supply of progesterone is available. The histological changes that occur as a result of endometrial traumatization have been described in the macaque monkey by Rossman (1941). Dawson and Kosters (1944) have shown that in the cat the presence of fertilised ova is not essential for the induction of pre-implantation changes in the endometrium. Recently, Blandau (1947) has shown that there is a definite species difference in the endometrial response to glass or paraffin beads, approximately the size of the blastocyst, inserted into the uterus. In the rat, decidual crypts were formed and the beads became implanted in a similar manner to ova, whereas, in the guinea-pig the beads did not penetrate the uterine epithelium and only a minimal decidual reaction was induced.

It will thus be seen that the precise rôles of egg and endometrium during implantation are unknown. It is probable that the egg exerts some influence, possibly both chemical and physical, on the endometrium. It seems reasonable to presume "that a cytolytic substance is responsible for the actual erosion at the site of contact" (*see* Wislocki and Streeter, 1938). This conclusion is strongly supported by the endometrial changes at the site of formation of the future secondary placenta in the macaque monkey (*see* p. 79). There is, however, as Fawcett *et al.* (1947) have recently stressed "very little proof that the trophoblast does actually secrete cytolytic enzymes." These workers, who studied the development of mouse ova in the anterior chamber of the eye and in the peritoneal cavity, stress the viewpoint that the actual mechanism of implantation will probably never be found by strict adherence to either of the extreme views that attribute it chiefly to trophoblastic or decidual activity. They write:—"The ovum and the uterine

mucosa no doubt have overlapping rôles. The endometrium is able to "implant" an inert object. The ovum is also capable of imbedding itself in extrauterine sites. Experimentally induced deciduomata may bleed into the uterine lumen. The trophoblast can also cause an extravasation of blood when transplanted outside the reproductive tract. It is probably an over-simplification of the complex process of implantation to assign separate rôles to the maternal and foetal parts. The individual potentialities of the ovum and the uterine mucosa should not be thought of as mutually exclusive but mutually supporting, and neither is 'chiefly' responsible for implantation."

VIII. IMPLANTATION (EMBEDDING OR NIDATION OF THE MAMMALIAN EGG)

During the period in which the blastocyst is forming the zona pellucida becomes thinned and is eventually dissolved so that the trophoblastic cells can come into

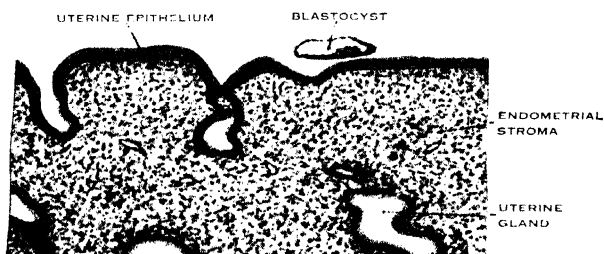


Fig. 14. 24—A section of the 9th-day macaque blastocyst at the early stage of attachment. In this section the blastocyst appears to be free. $\times 60$. (From Hamilton, Boyd and Mossman, 1945, after Wislocki and Streeter, 1938.)

direct contact with the maternal endometrium (Fig. 14. 24). The trophoblastic wall of the blastocyst should be regarded as a membrane, the main function of which is to supply nutritive material for the immediate needs of the egg. No part of the trophoblast becomes incorporated in the definitive embryo; it is an extra-embryonic ectodermal layer.

Bonnet (1882) has described three main types of implantation—central, eccentric and interstitial—depending on the relationship of the blastocyst to the uterine cavity. Although this classification is useful, it must be stressed that the mode of implantation has no relationship to the ultimate nature and shape of the definitive placenta. In particular, the type of implantation has no obvious association with the amount and nature of uterine mucosal and decidual reaction and destruction. Thus implantation in the pig and rabbit are both central and superficial; in the former, however, the definitive placenta is of the epithelio-chorial type (p. 65), whereas, that of the latter is of the haemochorial type and may even be haemoendothelial (p. 72).

The way in which the blastocyst implants shows many variations in different mammalian orders; even in the same order and family the depth of implantation may be variable (see later).

In some orders the blastocyst remains in the uterine cavity and eventually expands to fill the lumen: such implantation is central. This type of implantation is found in ungulates, carnivores, the leporid family of rodents, the Lemuroidea, Tarsoidea, platyrrhine and catarrhine monkeys, some insectivores (e.g. *Sorex* and *Chrysochloris*), some edentates and some of the vespertilionid bats.

In other mammalian orders the blastocyst remains small and comes to occupy a small diverticulum or cleft of the uterine lumen; this is eccentric implantation. After implantation, when the placenta develops, the original lumen is obliterated. Eccentric implantation is found in many rodents (*see p. 71*).

In still other mammals, the blastocyst implants by passing through the uterine epithelium and becomes completely cut off from the uterine lumen. This type of implantation is interstitial. It is found in *Cavia* (guinea-pig), certain Chiroptera (e.g. the phyllostomid bats, *Glossophaga soricina*), *Erinaceus* (hedgehog) amongst the insectivores, the chimpanzee and man.

The site where the blastocyst becomes implanted shows species differences. It may be on the antimesometrial side of the uterus, as in most rodents and most insectivores, or on the mesometrial side, as in *Pteropus edulus* (a megachiropteran) and in *Tarsius* (*see Mossman, 1937, for details*). In certain species the implantation is orthomesometrial (i.e. alternating ventrally and dorsally), as in *Centetes* and *Hemientetes* (Goetz, 1937).

The orientation of the embryonic disc within the uterus shows species differences—it may be mesometrial, as in rodents, antimesometrial, as in the vespertilionid bats and some insectivores, or lateral, as in *Chrysochloris* and *Molossus* (Fig. 14. 25). The significance of this orientation is not understood, but it is related in some way to the point of first attachment of the trophoblast to the endometrium and the subsequent position of the placenta. In rodents the first attachment is antimesometrial and the placenta is mesometrially situated. In some bats and in the insectivores the attachment is antimesometrial and the placenta also develops in this region, i.e. in close proximity to the embryonic disc. In the Megachiroptera (*Pteropus edulus*) the first attachment is mesometrial and the placenta is also in this position. In *Macacus rhesus* and in man the part of the trophoblast which establishes the first attachment overlies the inner cell mass and is on either the dorsal or ventral uterine wall (positions corresponding to the antimesometrial side of a bicornuate uterus).

Ungulata

The pig and sheep have been chosen as the ungulate prototypes. In the former the definitive placenta is epithelio-chorial (Chap. 15), while in the latter it is syndesmochorial with cotyledons (Chap. 15). There are transitional stages between these two types, e.g. in the Mexican deer (*Cervus mexicanus*) the definitive placenta in some parts is epithelio-chorial and in other parts syndesmochorial.

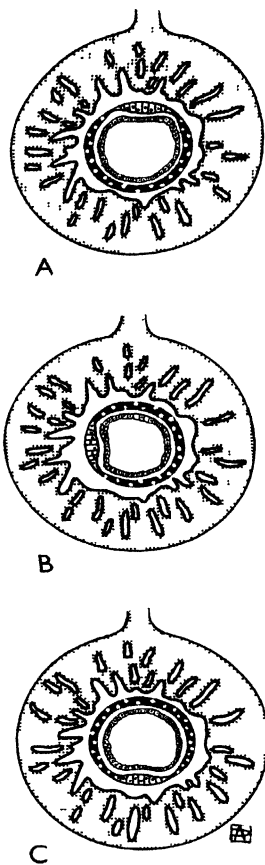


Fig. 14. 25—Diagrams to show schematic representation of possible orientation of the blastocyst with reference to the uterus and mesometrium. A. Mesometrial orientation of embryonic disc. B. Lateral orientation. C. Anti-mesometrial orientation.

In the sheep (Assheton, 1906) and in the pig (Corner, 1921) the histological changes in the uterus during the first 15 days following oestrus are similar in pregnant and non-pregnant animals. By the 7th or 8th day after fertilisation,

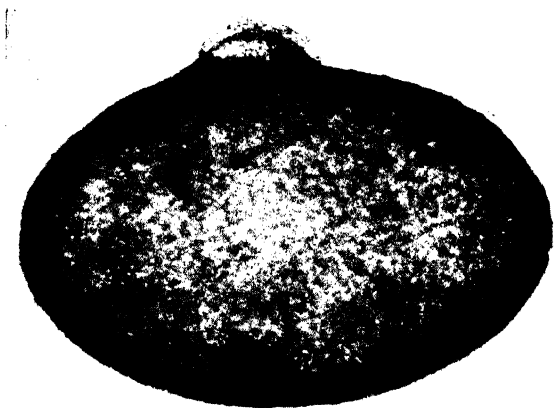


Fig. 14. 26—Living sheep blastocyst 12 days post insemination. The embryonic disc is raised above the general level of the trophoblast. $\times 48$.

the blastocysts have become enlarged (Fig. 14. 26) due to the accumulation of fluid in the blastocyst cavity (Fig. 14. 27). The epithelium of the uterus is still columnar. In the sheep the zona pellucida disappears about the 10th day (earlier in the pig, Heuser and Streeter, 1929), so that the trophoblastic cells now come directly into contact with the uterine epithelium. The trophoblastic cells, in spite of the rapid increase in size of the blastocyst cavity, have changed from the flattened cells of the 8th day stage to a cuboidal shape (Fig. 14. 28). The inner cell mass

(embryonic knot) becomes intercalated in the wall of the trophoblast and is soon converted into a flattened embryonic disc. The endodermal cells form a layer of flattened cells on the deep aspect of the disc and eventually spread round the inner

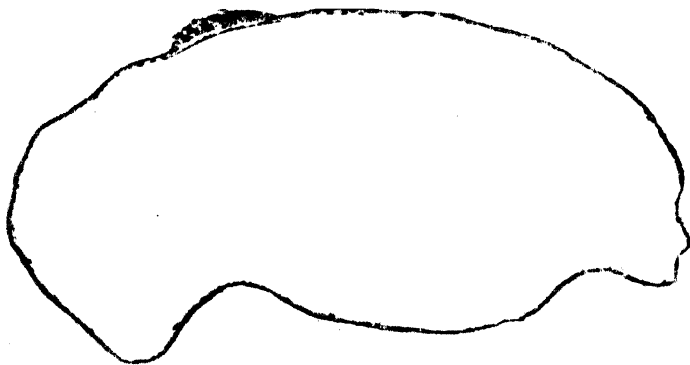


Fig. 14. 27—Section through blastocyst shown in Fig. 14. 26. The endoderm can be seen on the deep aspect of the exposed embryonic ectoderm. $\times C 86$.

aspect of the trophoblast to form the yolk sac (Figs. 14. 23, 27 and 28), so that the blastocyst is now bilaminar. The non-embryonic part of the blastocyst wall can now be called the bilaminar omphalopleure.

In the pig the wall of the blastocyst becomes folded, due, according to Heuser and Streeter, to the fact that the trophoblastic expansion outstrips the accumulation

of fluid in the blastocyst cavity. In the sheep this collapse has not been observed by us until a later stage. In the pig the blastocyst begins to elongate at the end of the 7th day (Green and Winters, 1946). In the sheep, however, this elongation does not occur until the 12th day (Hamilton *et al.*, 1943). By the 14th day the blastocyst, now often referred to as the chorionic vesicle, forms an elongated sac (Fig. 14.30). Mesoderm from the primitive streak passes laterally between trophoblast and endoderm, to line the former and establish the chorion (Figs. 14.23 and 29).

In the pig, by the 11th day the chorion is in partial apposition with the uterine epithelium, which has developed irregular edges. From now onwards the histological changes of the endometrium differ in the pregnant and non-pregnant animal, so that by the 20th day in the former the uterine cavity is lined with cuboidal cells which show irregularities of their free surface, whereas in the non-pregnant animal the pro-oestrous changes are manifest. The irregular surface of the epithelial cells, Corner believes, assists in the attachment of the chorion to epithelium. The epithelial cells do not degenerate as a result of the attachment of the chorion to them, but soon become columnar again (*see* Ch. 15). During the later stages of implantation when the embryo has about six somites, the allantois appears as an evagination of the hindgut. The significance of this structure is discussed on p. 101.

Implantation in the sheep differs considerably from that of the pig. The uterine mucosa possesses caruncles (cotyledonary burrs) (Figs. 14.32 and 33) which are present from the foetal period and are independent of oestrus. These caruncles are traversed by numerous

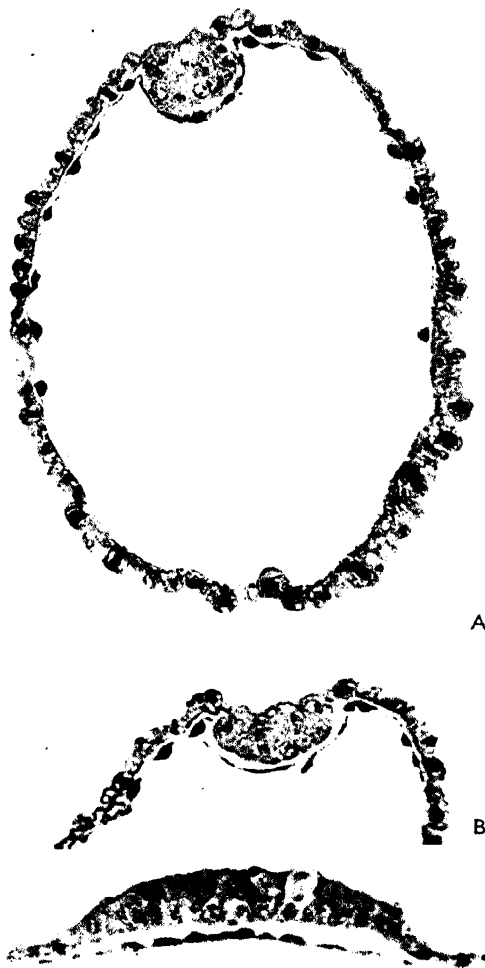


Fig. 14.28—A. Section of sheep blastocyst 10 days post insemination. The endoderm can be seen lining the upper half of the blastocyst cavity. $\times 230$. B. Section through inner cell mass region of another sheep blastocyst at the 10th day. The inner cell mass is becoming intercalated into the trophoblast. $\times 230$. C. Section of embryonic disc of a sheep blastocyst 12 days post insemination. The embryonic formative ectoderm is now bulging above the level of the adjacent trophoblast. $\times 280$. Compare with Fig. 14.26.

capillaries and are devoid of glands. They contain a varying amount of a melanin-like pigment. It is to these caruncles that the attachment of the chorion takes place. In the sheep, as in the pig, the chorion of the elongated blastocyst comes into contact with the uterine mucosa. At about the 18th day



Fig. 14. 29—Fixed and clear sheep blastocyst, early 13 days post insemination. The region of the inner cell mass and of the extra-embryonic mesoderm can be seen.
× 50.

the chorionic sac has expanded until it fills the uterine horn, or if there is only a single pregnancy, it may even pass into the other horn. With the expansion of the chorionic sac the trophoblast comes into contact with the epithelium over the caruncles. The caruncular epithelium is invaded and



Fig. 14. 30—The elongated sheep blastocyst (chorionic sac) of a late 13-day post insemination.
× 10.

eventually destroyed by the trophoblast; subsequently, the destruction and invasion extends until all the epithelium in contact with the chorion is destroyed. This type of implantation, in which there is destruction of the uterine epithelium, is often regarded as the first step in the elaboration of a more intimate relationship between chorion and maternal tissue than is found in the epithelio-chorial placenta.

At about the 15th day the allantois appears from the hindgut. It soon becomes bilobed (Fig. 14. 31B); later it expands and partially obliterates the chorionic cavity (Fig. 14. 33). For its further development *see* p. 101.



Fig. 14. 31—A. A 15-day sheep embryo with the chorion over the embryo removed. The amniotic cavity is shown and the remains of the chorio-amniotic junction. $\times 20$. B. Dorsal view of a somite sheep embryo (17 days post insemination) showing early development of allantois which appears as an anchor-shaped diverticulum at the caudal end. The anterior and posterior neuropores are still open and the amnio-chorionic junction is still visible. $\times 35$.

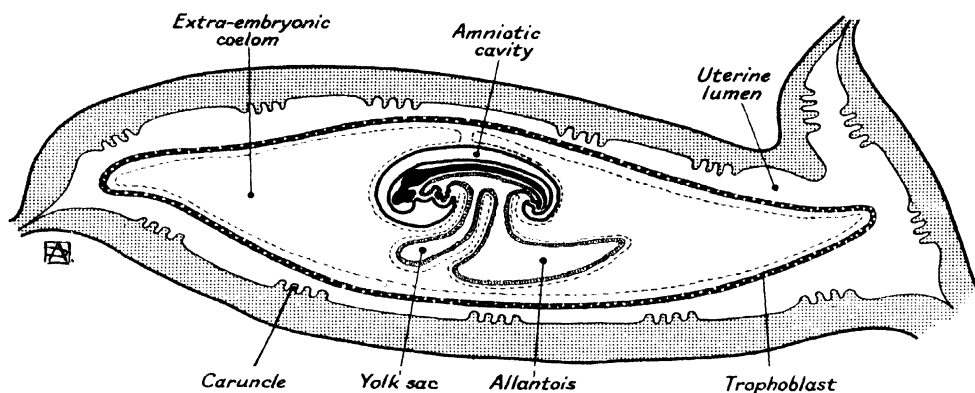


Fig. 14. 32—Scheme to show relationships at an early stage between the foetal membranes and the uterine cavity in the sheep. One chorionic sac is represented in the cavity of one uterine horn.

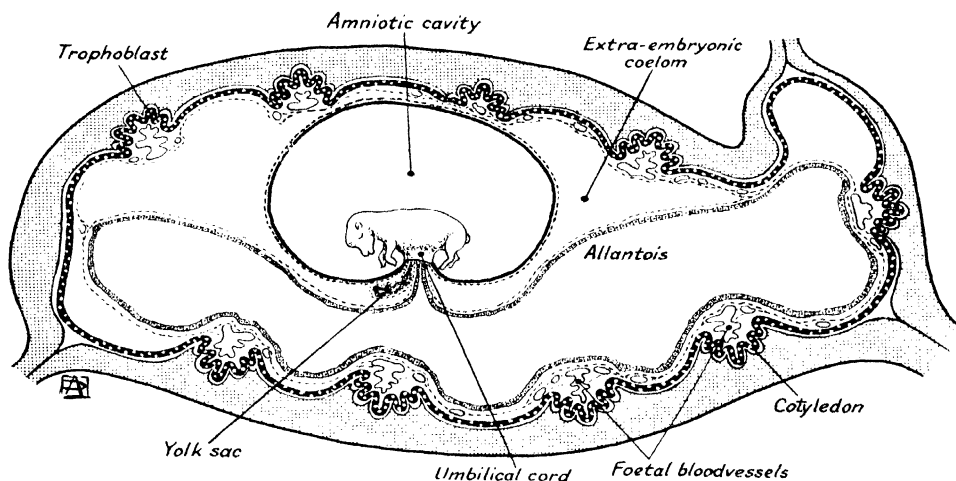


Fig. 14. 33—Scheme to show relationships at a later stage, between the foetal membranes and the uterine cavity in sheep.

Carnivora

In carnivores, detailed knowledge is only available for dog and cat, and is based on the work of Duval (1893, 1894 and 1895a), Bonnet (1897, 1901 and 1902), Schoenfeld (1903), and, more recently, Courrier and Gros (1932b). There are, however, scattered references to implantation in other carnivores and Hansson (1947) has recently given a full description of delayed implantation and associated phenomena in the mink. The oestrous cycle has been described in the cat by Courrier and Gros (1932a) and Dawson and Kusters (1944); the latter investigators also give an account of the endometrial distribution of glycogen and fat. The oestrous cycle of the dog has been described by Evans and Cole (1931), and the histological changes are essentially similar to those that occur in the cat. Evans

and Cole found that the changes in the non-pregnant and pregnant animal were similar up to and including the fourteenth day following ovulation.

During the luteal phase in the cat, Courrier and Gros (1932a) have shown that there are two stages ; for the first 7 days there is intense glandular activity, as shown by the hypertrophy of the cells. This stage is followed by a second stage characterised by the proliferation of the superficial epithelium. This proliferation results in the formation of numerous folds giving the epithelium a dentate appearance. The blastocyst of the cat reaches the uterus on the 7th or 8th day after mating (Manwell and Wickens, 1928) and remains free until the 13th or 14th day (data of Courrier and Gros, 1932b, *see also* Dawson and Kusters, 1944, for discussion). During the period when the blastocyst is free in the uterus, the actively secreting glands provide "embryotrophe" for the growing blastocyst. By the time that the blastocyst is ready to implant, the inner cell mass has become intercalated into the trophoblast, and the embryonic ectodermal cells form a flattened embryonic disc, as in ungulates. The endoderm forms a complete lining for the blastocyst. During the period of attachment of the trophoblast to the epithelium the immediately underlying stroma forms a decidual layer containing an extensive capillary network. The surface epithelium at the site of attachment soon becomes flattened and its nuclei show degenerative changes. The trophoblastic cells proliferate and invade the epithelium. At the same time the crypts of the uterine glands are invaded by the proliferating trophoblast, with the result that the cells of the glands unite to form a syncytium.

Eventually the epithelial lining of the uterus and that of the crypts of the uterine glands are replaced by plasmodi-trophoblast, so that the latter lies in direct contact with the decidual layer. This plasmodi-trophoblast proliferates and eventually surrounds the maternal capillaries of the decidual layer.

Rodentia

Only a very brief summary can be given of the implantation of rodents ; for details reference should be made to the works of Duval (1891), Burckhard (1901), Maclaren (1926), Wilson (1928), Sansom and Hill (1931), Maclaren and Bryce (1933), Mossman (1937), Mossman and Weisfeldt (1939), Mossman and Hisaw (1940) and Nielson (1940).

In this order implantation varies from superficial in the rabbit to completely interstitial in the guinea-pig. Intermediate types are shown by *Geomys* (pocket gopher), in which it is incompletely interstitial, *Citellus tridecemlineatus* (ground squirrel or spermophile) and *Castor* (beaver), where it is partly interstitial, and in *Dipodomys* (kangaroo rat) and *Mus musculus* (house mouse), where it is almost completely interstitial.

The rodents show a number of unusual developmental features—e.g. inversion or entopy of the germ layers and ultimate disappearance in most families of the abembryonic part of the trophoblast and yolk sac. With the exception of the blastocyst of the rabbit, which rapidly expands to fill the uterine lumen at the site of implantation, the blastocysts of rodents so far investigated remain small until after implantation. Before implantation they come to lie either in the anti-mesometrial part of the lumen or in small crypts in this region (Fig. 14, 34B). The embryonic disc is always orientated towards the mesometrial side and the blasto-

cyst usually makes first contact with the uterine epithelium by its abembryonic pole (Fig. 14, 34A).

In the rabbit, during the 6th and 7th days after copulation, the blastocyst expands to fill the antimesometrial part of the uterine lumen, the folds of which disappear. The epithelial cells lining the uterus and glands lose their distinct outline and their nuclei undergo fragmentation. The trophoblast becomes attached to and eventually invades and destroys the epithelial lining, coming into relation with the subepithelial tissues and blood vessels. The vessels are invaded by the trophoblast on the 9th day, so that maternal blood is in contact with the trophoblast.

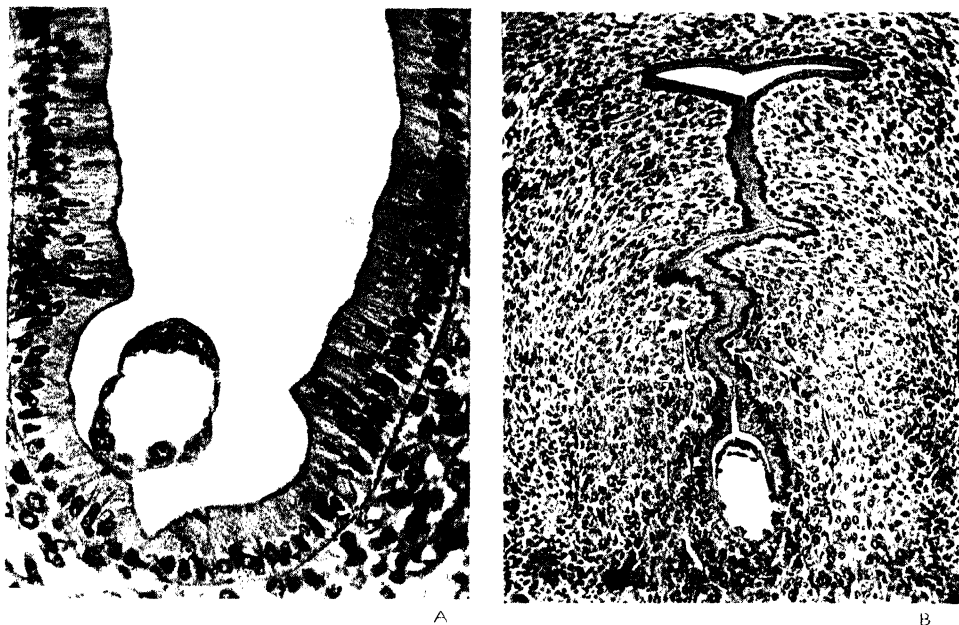


Fig. 14, 34—A. Transverse section of the antimesometrial portion of the uterine cavity in the golden hamster, *Cricetus auratus*, to show early attachment of the blastocyst. $\times 320$.
B. Transverse section through complete uterine cavity to show blastocyst at the implantation site. $\times 80$.

The trophoblastic cells in relationship with two mesometrial folds undergo marked proliferation to form a horseshoe-shaped ectoplacenta. During the rapid expansion of the blastocyst the embryonic ectoderm is exposed on the surface of the blastocyst, as in the ungulates and carnivores. (For the passage of maternal proteins through the trophoblast in the rabbit see Brambell and Mills, 1947a.)

In the ground squirrel (Mossman and Weisfeldt, 1939) the trophoblast thickens at the implantation pole to form a plasmodi-trophoblast. At a small area on the antimesometrial side of the uterine wall the trophoblast penetrates to the endometrial stroma, without apparently destroying the epithelial cells. After this penetration, the trophoblast spreads out centrifugally with root-like processes under the epithelium. This trophoblastic proliferation now becomes hollowed out to form a cup-like diverticulum and the processes disappear. At the same time

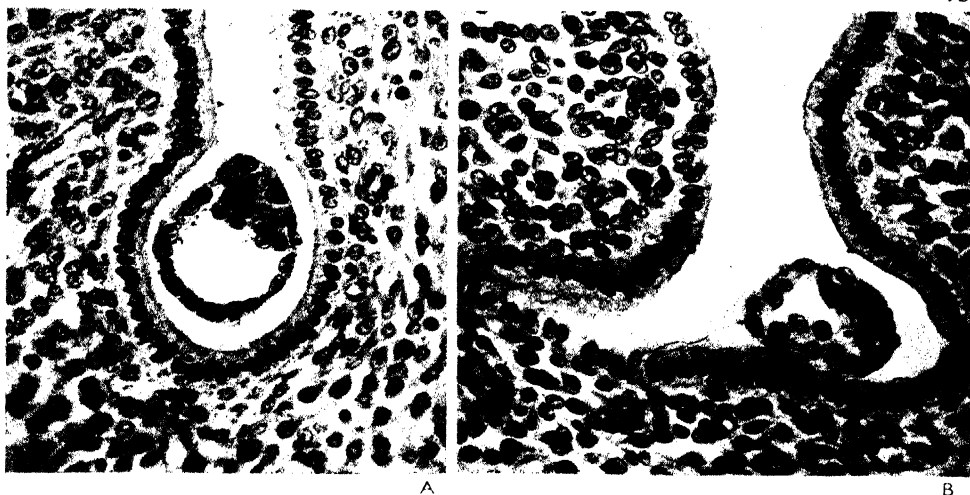


Fig. 14. 35—A. Free blastocyst of the mouse in the antimesometrial crypt of the uterine cavity. B. Early attachment of the mouse blastocyst to uterine epithelium in the crypt. (Reproduced by the courtesy of Professor E. C. Amoroso.)

as the plasmodi-trophoblast is becoming converted into a cup-like diverticulum the trophoblast over most of the blastocyst wall becomes attached to the uterine epithelium. In the region of attachment the uterine epithelium gradually disappears. As the endoderm has grown round the inner aspect of the trophoblast a bilaminar yolk-sac placenta is formed. It is through the yolk-sac placenta that the embryo at this stage derives its nutrition from the mother. The trophoblast at the sides of the embryonic disc becomes thickened, and, at the same time, the mesoderm passes between the trophoblast and endoderm for a short distance. This mesoderm becomes vascularised so that a chorio-vitelline placenta is formed.

In the mouse (Duval, 1891; Burckhard, 1901; Sobotta, 1903; Bloch, 1939; and Snell, 1941) the blastocyst comes to occupy a crypt in the antimesometrial side of the uterine lumen on the 5th day after mating (Fig. 14. 35). Within a few hours after the blastocyst has come into contact with the endometrium, degenerative changes, as evidenced by the loosening of the epithelium, become apparent (Fig. 14. 36). Sobotta showed that the epithelial cells become flattened at the area of contact.

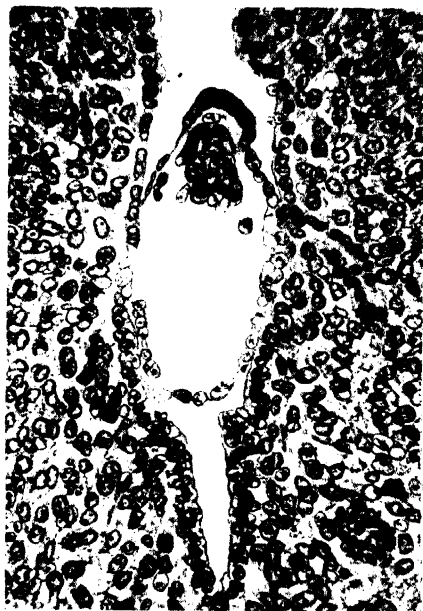


Fig. 14. 36—Section through implanting mouse blastocyst during the 5th day. Portion of the antimesometric part of the lumen is still present. (Reproduced by the courtesy of Professor E. C. Amoroso.)

At the same time as the epithelial cells are being destroyed by the trophoblast, the underlying stroma cells become enlarged (decidual reaction, *see* p. 63) and the endometrium becomes oedematous. The degenerating epithelial cells

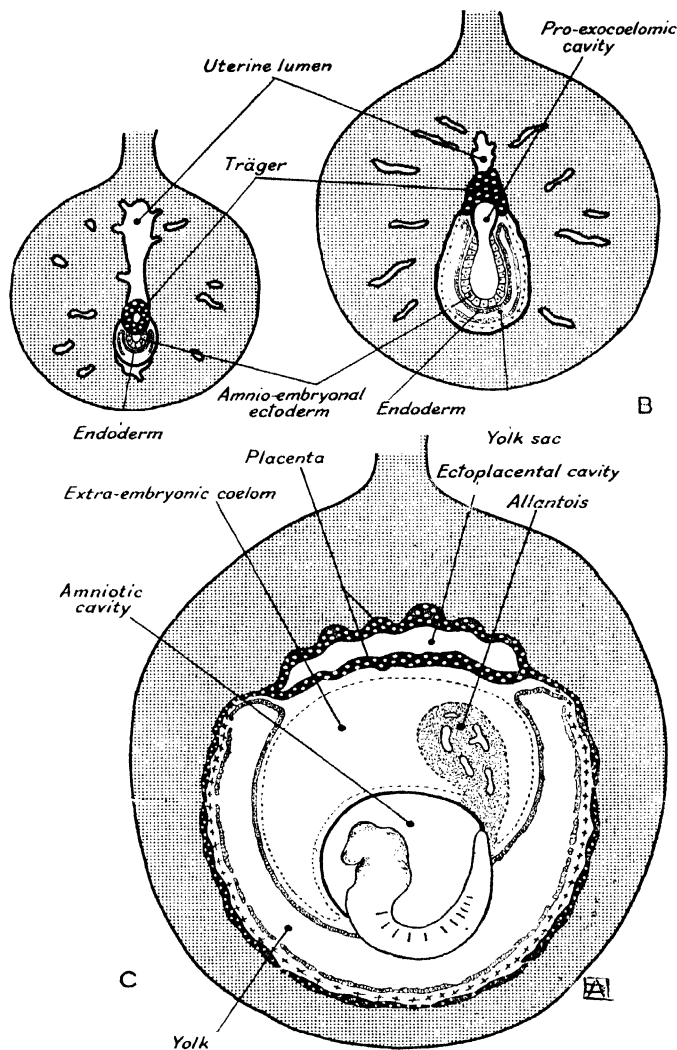


Fig. 14. 37.—Schematic transverse sections to show the implantation and placentation of the mouse. Reichert's membrane is shown by crosses.

serve as a source of food for the blastocyst ("histiotrophe" of Grosser). As soon as implantation is effected, there is marked growth of the adjacent uterine tissues, especially at the sides of the implantation site, to form the decidual cushions. These cushions fuse with each other on the mesometrial side of the trophoblast

(ectoplacental cone or *träger*) covering the differentiating inner cell mass and separating the trophoblast from the mesometrial part of the uterine lumen. During implantation the cells on the deep aspect of the inner cell mass differentiate to form the endoderm and soon spread round the inner side of the trophoblast to form the yolk sac (Figs. 14. 36 and 37). With the expansion of the decidual swelling towards the mesometrial side of the lumen, the ectoderm of the inner cell mass, together with the overlying endodermal cells, forms a cylinder: in this way the endoderm comes to surround the ectoderm; thus there is inversion of the germ layers. Later the ectoderm of the distal part of the cylinder is separated from the ectoplacental cone by a cavity, the pro-exocoelomic cavity (Fig. 14. 37B). Still later this cavity becomes lined by mesoderm to form the extra-embryonic coelom (Fig. 14. 37C). The trophoblastic cells, especially those of the ectoplacental cone, invade and destroy the surrounding endometrial tissues and eventually erode the maternal capillaries so that maternal blood corpuscles come into contact with the trophoblast. These blood cells are ingested by the trophoblast and form a "haemotrophe" for the embryo.

The early implantation of the guinea-pig has been studied by a number of investigators—von Spee (1901), Maclaren (1926), Sansom and Hill (1931), Maclaren and Bryce (1933), and Pytler and Strasser (1925). The precise method of implantation has been the subject of considerable discussion. See Wilson (1928) for a critical review.

The guinea-pig blastocyst, like that of the mouse, comes to lie in the antimesometrial part of the uterine cavity. It is composed of a covering layer of trophoblast and an inner cell mass. The abembryonic pole of the blastocyst shows a conspicuous thickening

During the 6th day after mating, the thickened trophoblast comes into contact with and destroys the uterine epithelium. Through the gap in the epithelium the blastocyst passes into the endometrial stroma and soon extends below the margins of the epithelium at the gap in the uterine wall. Eventually, the blastocyst comes to lie entirely within the uterine stroma and becomes more deeply implanted as the subepithelial tissues are invaded by the advancing trophoblast (Fig. 14. 38).

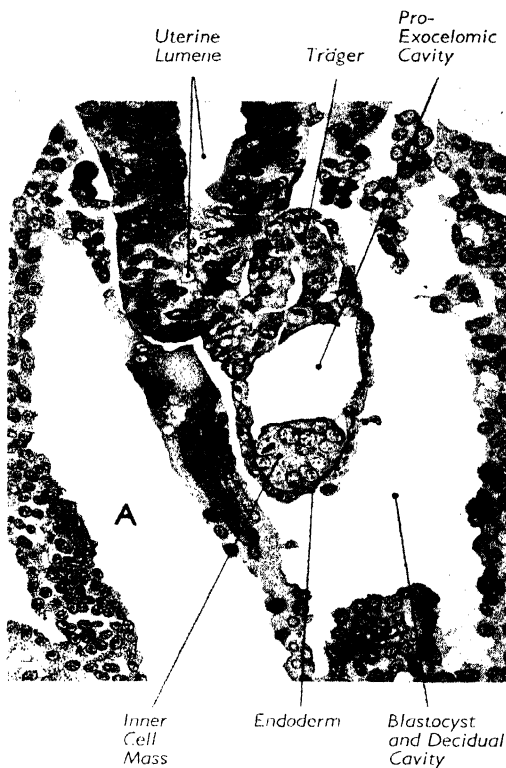


Fig. 14. 38—A section through implanting blastocyst of guinea-pig. A = Artefact space. (Reproduced by the courtesy of the Royal Society of Edinburgh.)

The margins of the gap gradually grow over the upper or placental pole of the blastocyst until finally the epithelium is re-established at the original site of implantation. When finally implanted the blastocyst occupies a subepithelial position with an upper or placental pole covered with ectoplacental trophoblast. This trophoblast is continuous with the remainder of the trophoblastic wall which gradually becomes attenuated as it invades the endometrial stroma.

Primates

In this order there is great diversity in the manner in which the blastocyst comes to be attached to, and finally implanted in, the uterine mucosa. Hill (1932) has grouped the order into four subdivisions—Lemuroid, Tarsioid, Pithecoïd and Anthropoid. The Pithecoïd group contains the platyrrhine and catarrhine monkeys, while the Anthropoid group includes the anthropoid apes and man. The Primates, in Hill's opinion, show more adaptations during implantation and later developmental stages than any other mammalian order.

The pioneer work on primate development was done by Selenka and Hubrecht. Since their investigations valuable contributions have been made by many other embryologists. This work has culminated in the contributions made by the investigators in the Carnegie Institute, Embryology Department, under the direction of Professor G. L. Streeter and his successor, Professor G. W. Corner.

Lemuroid stage

In *Loris*, which has been investigated by Hubrecht and Keibel (1907), Hill *et al.* (1928), and Hill (1932), the blastocyst comes to lie in a bay of the uterine mucosa

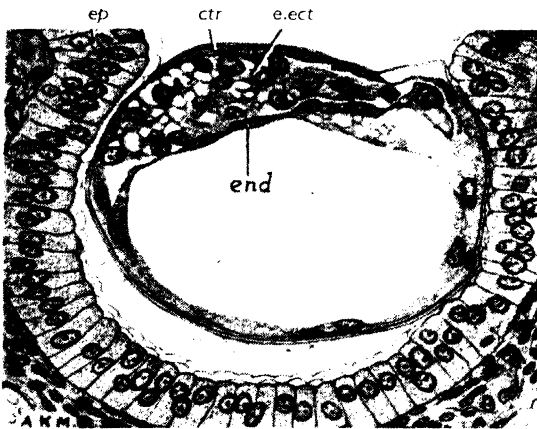


Fig. 14. 39—Section of the blastocyst of *Loris* occupying a crypt in the endometrium. (Reproduced by the courtesy of Professor J. P. Hill and the Royal Society.)

(Fig. 14. 39). It expands rapidly and soon fills the transverse diameter of the uterine lumen.¹ The columnar ectodermal cells become intercalated in the trophoblastic wall and the endoderm grows round the inner aspect of the trophoblast to form a yolk sac, as in ungulates and carnivores. Hill (1932) regards this rapid expansion of the blastocyst as a primitive feature, since the formation of a large yolk sac provides a cavity for the storage of nutritive material secreted by the uterine glands and absorbed by the trophoblast. The trophoblastic wall becomes

¹ In *Galago demidoffi* (Gerard, 1932) the appearances are quite different from the typical lemur. Implantation is at first interstitial, with embryonic ectoderm exposed and in contact with uterine tissues; later the capsularis disappears so that the implantation becomes secondarily superficial. *Galago* shows a further remarkable feature in that the extra-embryonic endoderm is mesenchymatous in appearance.

attached to the uterine epithelium in an unusual manner. The outer cuticular border of the cells forms "thin flanges" which penetrate for some distance between the uterine epithelial cells. In addition, parts of the trophoblast become thickened to form "absorptive areas." These areas are developed in relationship with the common openings of groups of uterine glands. The extension of extra-embryonic mesoderm from the primitive streak soon separates the endoderm from the trophoblast. Before the mesoderm splits into somatopleuric and splanchnopleuric layers, vessels are formed in it so that a temporary yolk sac, or omphalopleuric placenta, is formed. The development of the extra-embryonic coelom separates the yolk sac completely from the chorion, a distinctive primate feature.

Tarsioid stage

This stage is represented by *Tarsius spectrum*, the development of which has been described in a comprehensive series of papers by Hubrecht (1896, 1898 and 1902), and more recently reviewed by Hill (1919 and 1932). At the time of attachment the blastocyst consists of an outer trophoblastic layer and the inner cell mass, which is differentiating into ectodermal and endodermal layers. The blastocyst is attached by its abembryonic pole to the uterine epithelium on the mesometrial side of the uterus by a number of "sprout-like" outgrowths, which penetrate the epithelium and terminate in the subepithelial periglandular tissue. This initial mode of attachment is essentially similar to that in the macaque monkey (see later) and very different from that in *Loris*. In the region of attachment, the uterine epithelium undergoes marked hypertrophy, the cells become narrowed and elongated, with the nuclei crowded together. The epithelium of the glands at the site of attachment proliferates so that their lumen is occluded. Soon after attachment the blastocyst expands rapidly and the trophoblast covering the ectoderm ruptures and disappears, exposing the ectoderm as a flattened disc on the surface.

From the posterior margin of the ectodermal disc extra-embryonic mesoderm is developed precociously and spreads between the trophoblast and the yolk-sac endoderm in the posterior half of the blastocyst. The mesoderm splits to form the extra-embryonic coelom, except in the region of the embryonic disc, where it becomes the primordium of the connecting stalk. Further development of the extra-embryonic mesoderm and coelom completely separates the yolk sac from the chorion. This precocious formation of mesoderm in *Tarsius* anticipates conditions characteristic of the higher primates (see p. 85). Later, the primordium of the connecting stalk is augmented by mesoderm from the primitive streak. This mesoderm grows between the mesothelial layer of the coelom and the trophoblast to reach the chorion at the site of the future placenta, and into this mesoderm the allantois grows. Hill (1919) points out that the early appearance of the coelom in the mesoderm prevents the establishment of a yolk sac placenta vascularised from the vitelline vessels as in the lemurs. The absence of a yolk-sac placenta calls for an acceleration in the development of a connecting stalk and the growth into it of the allantois, in order that the placental area of the chorion may be rapidly vascularised. In later stages of implantation the trophoblast develops a mass of parenchymatous tissue. To this mass Hubrecht gave the name "trophospongia" and considered that it was of maternal origin. A comparable formation of an epithelial plaque of proliferated maternal epithelium in the macaque monkey (see p. 79) would seem to support Hubrecht's hypothesis. Hill (1932) on the

other hand is of the opinion that it is of trophoblastic origin. The final verdict must be postponed until more material becomes available.

Pithecoïd stage

Reviews of the literature on the implantation of the platyrrhine and catarrhine monkeys have been given by Hill (1932), Mossman (1937), and Wislocki and Streeter (1938). The following description is mainly based on the investigations of Wislocki and Streeter (1938) and Heuser and Streeter (1941).



Fig. 14. 40—Surface view of an 8-9 day implanting blastocyst of the macaque monkey. $\times 35$. (From Hamilton, Boyd and Mossman, 1945. After Heuser and Streeter, 1941.)

The morula of the macaque monkey reaches the uterus on the 4th or 5th day after ovulation and soon becomes a blastocyst which remains free until the 9th day. It undergoes considerable expansion so that the zona pellucida becomes stretched and finally disappears during the early part of the 9th day.

Wislocki and Streeter describe three stages of implantation in the macaque monkey—(a) the prelacunar stage or period of trophoblastic lacunae; (b) the stage of trophoblastic lacunae; and (c) the villous stage. During the 9th day after ovulation the blastocyst attaches itself to the uterine epithelium by the fusion of some of the trophoblastic cells (attaching trophoblast) near the inner cell mass (Figs. 14.



Fig. 14. 41—Section of the early attachment stage of the trophoblast to the uterine epithelium in the 9th day macaque blastocyst. $\times 280$. (From Hamilton, Boyd and Mossman, 1945. After Wislocki and Streeter, 1938.)

40 and 41). There may, however, be more than one primary point of attachment (Fig. 14.42). At the time of attachment the epithelium exhibits no local reaction, but soon after the epithelial cells show degenerative changes evidenced by clumping of the nuclei and cytolysis of the cytoplasm. The inner cell mass at this stage consists of primitive formative cells, polar trophoblastic (attaching trophoblast) and primary endodermal cells. During the tenth day the trophoblastic cells penetrate between and destroy the epithelial cells, and come into contact with oedematous congested subepithelial stroma. The oedematous condition of the stroma provides nutritive material for the trophoblast as it thickens rapidly to become the trophoblastic plate. Some of the surface epithelium, adjacent to and in the neighbourhood of the trophoblastic plate, and the epithelium of the glands in the attachment zone, proliferate to form epithelial plaques which, for a time, constitute a border zone between the advancing trophoblast and the mucosa. Whether chemical or mechanical factors are responsible for the epithelial proliferation and the dilatation of the capillaries has not been determined.

The cells of the inner cell mass undergo a rearrangement. The formative cells become columnar and form the ectoderm of the embryonic disc (Fig. 14.43). The primary endodermal cells spread beyond the disc as a lining to the trophoblast. Only part of the primary endoderm is involved in the formation of the yolk sac (*see* p. 94 and discussion by Heuser and Streeter, 1941).

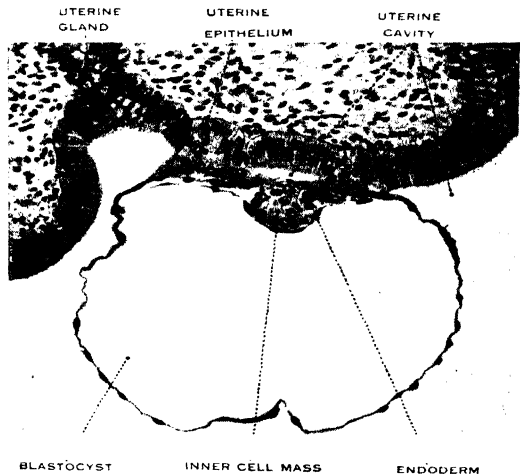


Fig. 14.42—Section of a later stage of the attachment of the trophoblast than in Fig. 14.41 (same blastocyst as illustrated in Fig. 14.40). The endoderm forms a layer underneath and beyond the edge of the inner cell mass. $\times 140$. (From Hamilton, Boyd and Mossman, 1945. After Heuser and Streeter, 1941.)

From the end of the 10th day the trophoblastic plate differentiates into an outer syncytium or plasmoditrophoblast and an inner cytotrophoblastic (Langhan's) layer. The syncytial layer grows rapidly and develops small clefts which enlarge into lacunae (Fig. 14.43). The dilated maternal capillaries in contact with the trophoblast rupture and the blood fills the lacunae.

The final phase of implantation is represented by the villous stage. From the 15th to the 17th day, the cytotrophoblast proliferates to form columns. These columns covered with syncytium constitute the villi. Later, mesenchyme and vasogenic tissue form a core in the villi (*see* Hertig, 1935).

In the macaque monkey, as in the majority of investigated platyrrhine and catarrhine monkeys, a secondary site of contact occurs at the abembryonic pole, between the trophoblast and the endometrium. As early as the 10th day epithelial proliferation occurs at this secondary site and the subsequent changes are essentially similar to those described at the primary site of attachment.

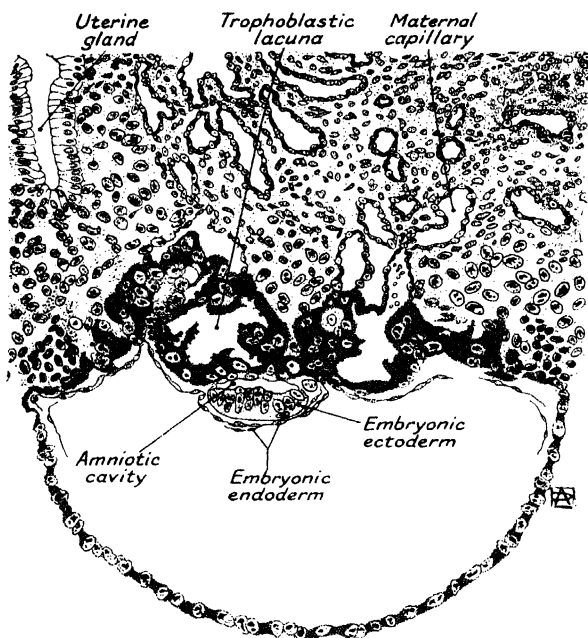


Fig. 14. 43—Semi-schematic section through the attached blastocyst of an 11-day monkey, based on the work of Wislocki and Streeter, 1938 and 1941.

Thus, in the later stages there are functional primary and secondary placentae (see Chap. 15).

Anthropoid stage

There is now an extensive literature on the early development and implantation of man.¹ The earliest stage of attachment of the human blastocyst has not been observed, nor has a free blastocyst as yet been recovered.² The recent publications of Hertig and Rock (1941 and 1945), Rock and Hertig (1942 and 1944), and Heuser, Rock and Hertig (1945) provide details of human implantation from 7½ to 16 days. Our knowledge of implantation in the anthropoid apes is limited to a description of a chimpanzee ovum of about 10½ days, described by Heuser (1940). The appearances are strikingly similar to the corresponding human stage.

In human subject.—Implantation in the human subject is interstitial and not superficial as in the macaque; in addition there is not the extensive epithelial reaction to the activities of the trophoblast. It is assumed that the human blastocyst becomes attached on the 6th day after ovulation. In the earliest stage of human implantation yet described (Hertig and Rock, 1941 and 1945) the blastocyst is already attached to the uterine epithelium and partially implanted 7 to 8

¹ A list of the early embryos has been recorded in tabular form by Hamilton, *et al.* (1945). See also Hamilton and Boyd, 1950.

² Hertig and Rock (personal communication) have secured a free blastocyst.

days after ovulation (Fig. 14. 44). The early mode of attachment of the trophoblast is probably similar to that of the macaque monkey. Whether the earlier attachment in man is due to the greater penetrative power of the human trophoblast (as maintained by Hill) or to the less resistant character of the endometrium to the invasive activities of the trophoblast (as suggested by Wislocki and Streeter, 1938), cannot yet be answered. Hertig and Rock (1945) have estimated that the stages of implantation in the macaque monkey from the 9th to 13th day, are comparable to those of the human from the 6th to 13th day. They suggested that "perhaps the interstitial implantation of the human blastocyst is less efficient than that of the monkey, and takes longer to produce the same functional results." The invading trophoblast in the

human $7\frac{1}{2}$ -day stage consists of a thick disc, which has differentiated into a syncytio-trophoblast and a cytotrophoblast. The unimplanted part of the blastocyst is composed of a thin mesothelial-like layer of trophoblast (Fig. 14. 44). There is as yet no attempt by the uterine epithelium to close the site of penetration. The endometrial stroma in contact with the trophoblast is swollen (pre-decidual reaction) and the cell nuclei show loss of chromatin. The inner cell mass consists of a layer of primitive ectodermal cells and a layer of endodermal cells. The amniotic cavity is represented by two clefts between the trophoblast and the ectodermal cells of the embryonic disc. In the next stage of development, at the 9th day, the blastocyst is only imperfectly covered with maternal epithelium. The syncytio-trophoblast forms an intercommunicating network of lacunae, containing a small amount of maternal blood, due to the invasion of the capillaries by the trophoblast. The superficial parts of the uterine glands in the implantation zone are surrounded by the invading trophoblast, so that the glandular epithelium shows varying degrees of degenerative changes. Primitive mesoblast is in the process of differentiating *in situ* from the cytotrophoblastic lining of the blastocyst. The endodermal cells form a layer underneath the ectoderm, which is now composed of columnar cells. Some of the cells at the periphery of the endoderm are in continuity with the mesoblastic cells which are becoming flattened to form the primordium of the exocoelomic or Heuser's membrane (*see* p. 94).

Within the next few days, from the 10th to the 15th day, the defect in the uterine epithelium is gradually closed by a coagulum of fibrin, and by the proliferation of the epithelium at the sides of the aperture of entry; the epithelium grows over

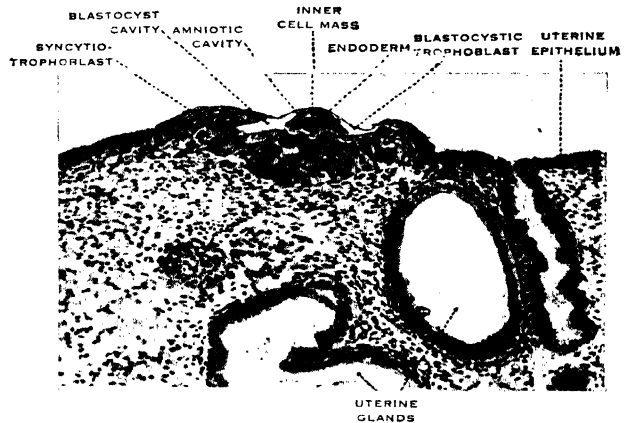


Fig. 14. 44—A section of a $7\frac{1}{2}$ -day human ovum partially implanted in secretory endometrium. The embryo is represented by the rounded mass of cells just above the trophoblastic mass and beneath the thin membranous portion of the collapsed blastocyst wall. $\times 100$. (From Hamilton, Boyd and Mossman, 1945. After Rock and Hertig.)

Within the next few days, from the 10th to the 15th day, the defect in the uterine epithelium is gradually closed by a coagulum of fibrin, and by the proliferation of the epithelium at the sides of the aperture of entry; the epithelium grows over

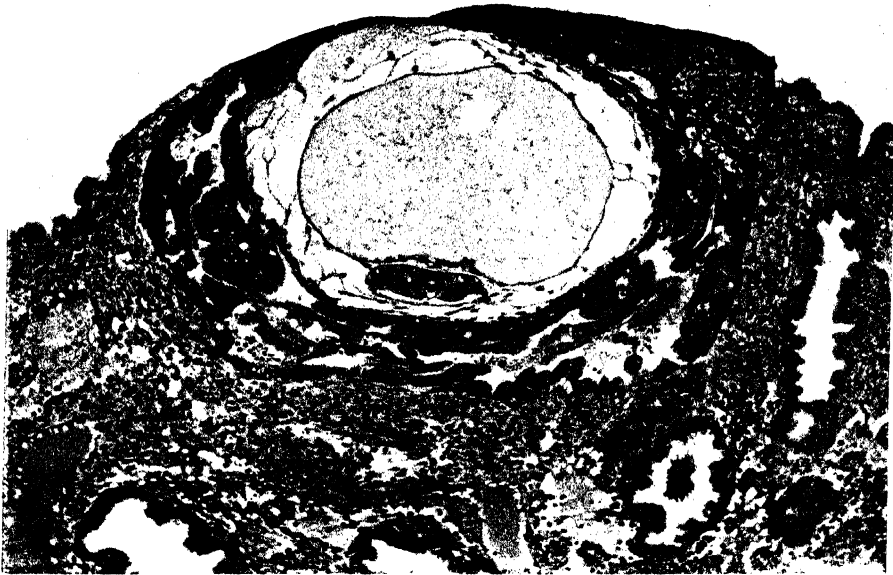


Fig. 14. 45—Photomicrograph of a human implantation of estimate age 12 days (Hertig and Rock). The syncytiotrophoblast has irregular lacunae containing some maternal blood. The cytotrophoblast consists of an inner irregular shell. The trophoblast at the abembryonic pole shows little differentiation and at one point is not yet covered with uterine epithelium. The inner aspect of the cytotrophoblast is lined with mesoderm which is condensed on its inner aspect to form a membrane, the exocoelomic or Heuser's membrane. The embryo consists of a thick layer of columnar embryonic ectoderm and a thinner layer of irregularly arranged polyhedral cells, the embryonic endoderm; the latter, at its edge, is continuous with the exocoelomic membrane. The space lined by the exocoelomic membrane and endoderm has been called the exocoelom by Hertig and Rock; in the present text it is referred to as the primitive yolk sac. A space, the amnion, lies between the mesoderm and embryonic ectoderm. $\times 100$. (From Hamilton, Boyd and Mossman, 1945. After Hertig and Rock, 1941.)



Fig. 14. 46—Surface view of the implantation site of a 12½-day human embryo. The mouths of the uterine glands are prominent. The ovum appears as a slightly raised area. $\times 6$. (From Hamilton, Boyd and Mossman, 1945. After Hertig and Rock, 1941.)

the fibrin, so that the blastocyst is completely enclosed in the endometrium (Figs. 14. 45 and 48), where it now produces a slight swelling (Fig. 14. 46). The endometrium can be subdivided into three topographically distinct portions, depending on its relation to the blastocyst. The portion on which the blastocyst is implanted is called the decidua basalis, that separating the blastocyst from the uterine lumen is the decidua capsularis, and that lining the rest of the uterus is the decidua parietalis. The trophoblast is not uniformly developed over the surface of the implanted blastocyst; it is thicker towards the basalis than towards the capsularis.

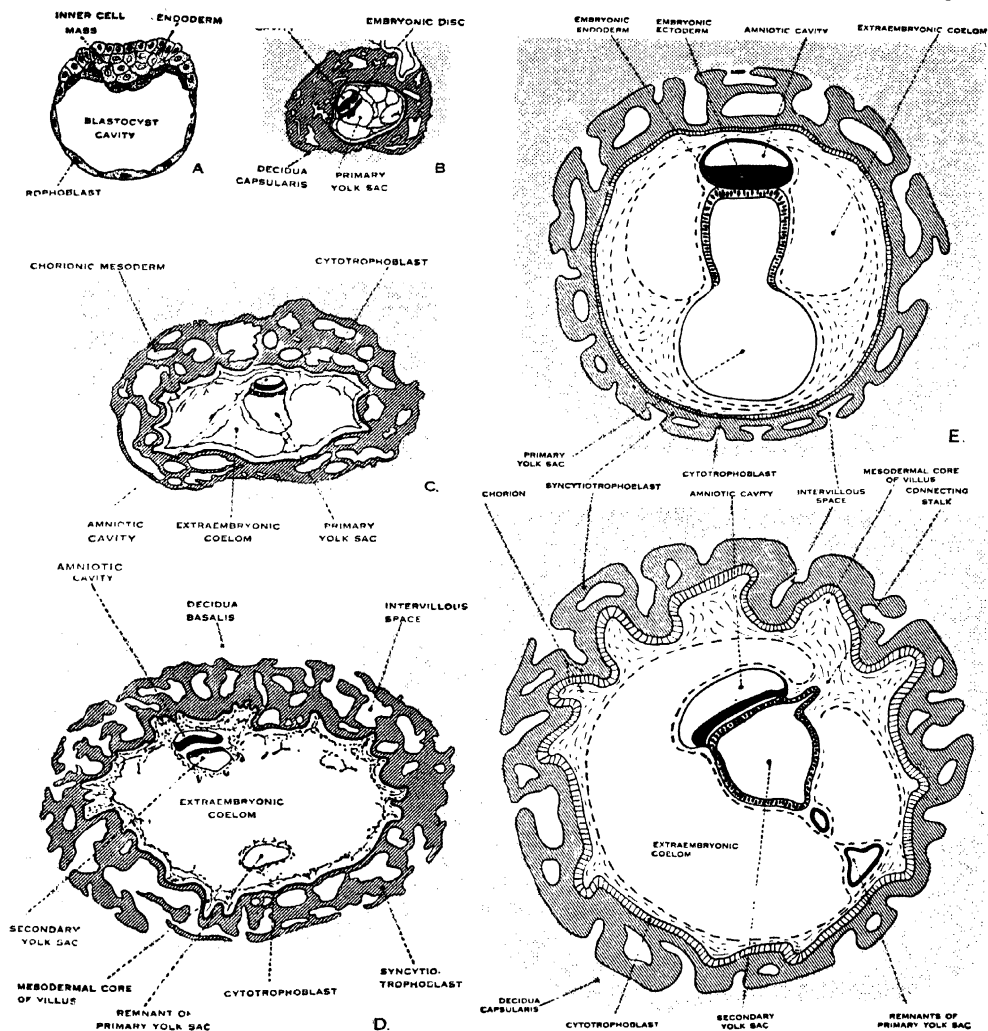


Fig. 14.47—Diagrams to show the development of the human yolk sac.

A. Hypothetical. B. Based on Carnegie ovum No. 7699. C. Based on the Werner (Stieve) ovum. D. Based on the Edwards-Jones-Brewer ovum. E. Hypothetical to show a process by which the large primordial yolk sac of earlier stages (C) may be converted into the smaller secondary yolk sac. F. Composite illustration based on several young human embryos to show the secondary yolk sac and the remnants of the primary yolk sac. (From Hamilton, Boyd and Mossman, 1945.)

From the 10th to 13th day, the embryonic disc is approximately circular in outline and is composed of columnar ectodermal cells with somewhat flattened endodermal cells (Figs. 14.45 and 47). At the edge of the disc the endodermal cells are continuous with a thin layer of mesothelial-like cells (the exocoelomic membrane). This membrane, together with the embryonic endoderm, encloses

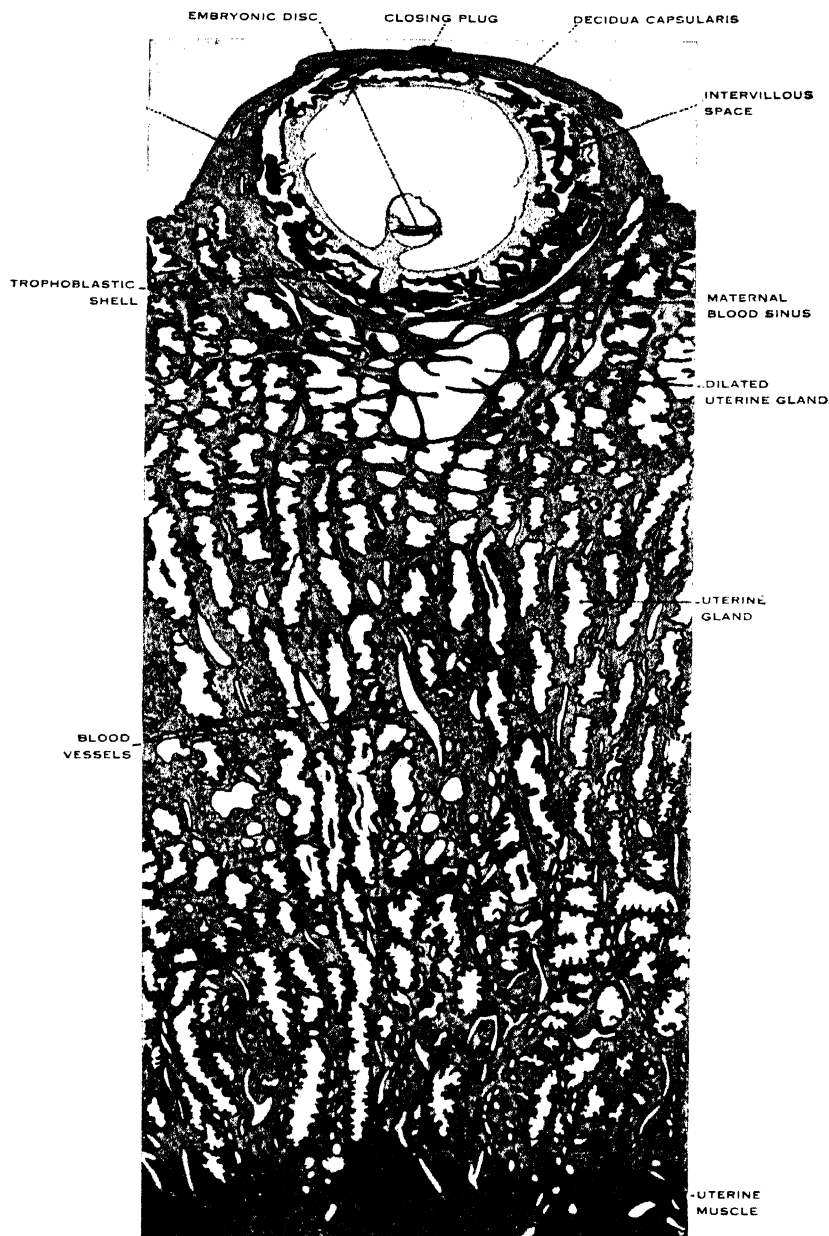


Fig. 14. 48—Drawing to show the relationships between the implanting chorionic vesicle and the endometrium at about the 15th day after fertilisation. The intrachorionic rudiment is attached to the inner aspect of the chorionic vesicle by the connecting stalk. Based on the Edwards-Jones-Brewer and Falkner embryos. $\times 20$.
(From Hamilton, Boyd and Mossman, 1945.)

the primary yolk sac (*see* p. 94). The tissue separating the primary yolk sac from the inner aspect of the cytotrophoblast is the primary extra-embryonic mesoderm. There has been much speculation and conjecture concerning the origin of this primary extra-embryonic mesoderm. Hill (1932) and Florian (1933) are of the opinion that it is precociously developed allantoic mesoderm, as in *Tarsius*, and that it arises from the ectoderm posterior to the site of the future cloacal membrane. In the human subject, as in the monkey, it develops before

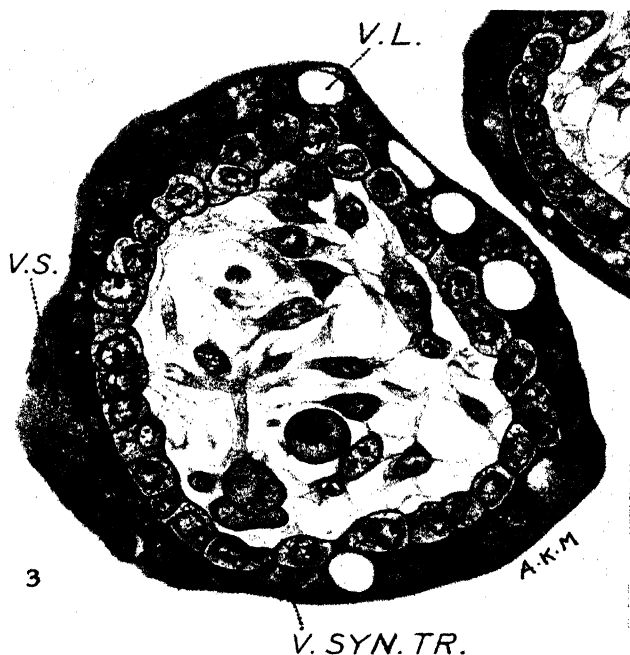


Fig. 14. 49—A transverse section of a villus of an 18-day embryo (Shaw). Blood vessels have not yet appeared in the villus. The syncytiotrophoblast shows the "brush border." $\times 550$. (From Hamilton, Boyd and Mossman, 1945. After Hamilton and Gladstone, 1942.)

the appearance of the primitive streak. The investigations of Hertig (1935), Wislocki and Streeter (1938), Heuser and Streeter (1941), and Hertig and Rock (1945), seem to indicate that this mesoderm is derived, in part at least, from the trophoblast.

At about the 13th and 14th days after ovulation, the syncytio-trophoblast is penetrated by columns of cytotrophoblast, which grow out and come into contact with the decidua. These columns are the cores of the primary villi (Fig. 14. 45). Almost as soon as a primary villus is formed, there is a concomitant extension into it of a secondary core of extra-embryonic mesenchyme to form a secondary villus (Figs. 14. 47, 48 and 49). An arterio-venous capillary loop is developed in the mesenchymal tissue of each secondary villus to form a tertiary villus. The arterio-venous loop becomes continuous with vessels which develop in the mesenchymal

lining of the cytotrophoblast and in that of the body stalk, through which they later connect with intra-embryonic vessels.

The cytotrophoblast columns are covered by the syncytium, except at their tips, where the cytotrophoblast extends through the syncytium and comes into direct

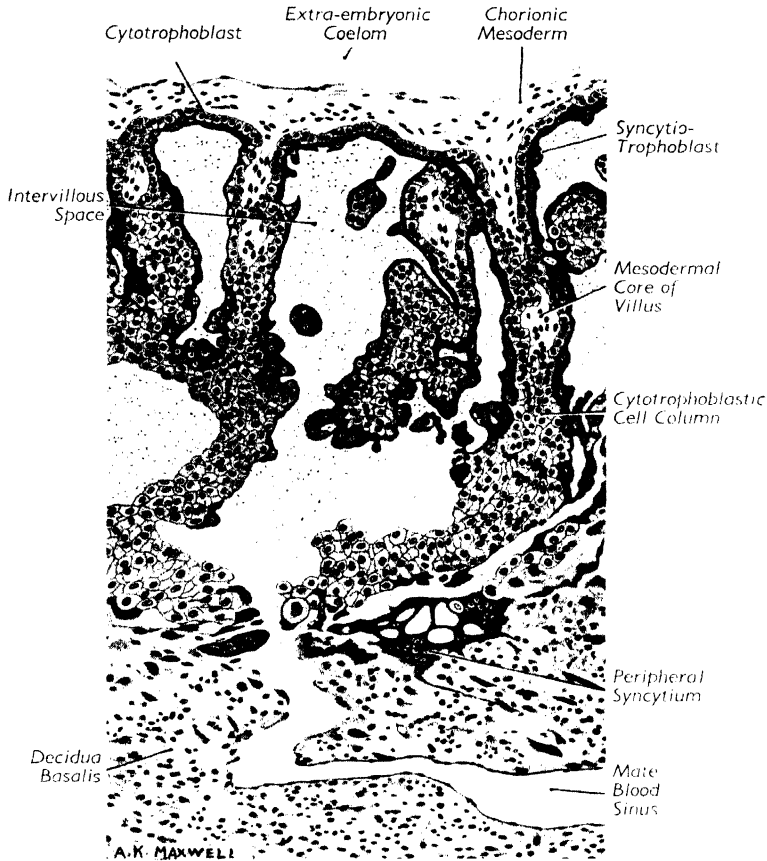


Fig. 14. 50 Section through a portion of the wall of the chorionic vesicle to show the method of attachment of the peripheral syncytium and cytotrophoblastic cell columns to the maternal decidua basalis and maternal blood sinus. $\times 100$. (From Hamilton, Boyd and Mossman, 1945. After Hill, 1932.)

contact with the decidua to form the trophoblastic shell (Fig. 14. 50). This arrangement anchors the chorion to the maternal tissue. From these main anchoring villi, secondary branching villi with free tips are developed. By the development of the extra-embryonic coelom the primary yolk sac becomes separated from the chorion, except in the region of the amnio-chorionic connexion, where the mesoderm remains unsplit to form the primordium of the connecting stalk.

In chimpanzee.—The chimpanzee ovum, which is estimated to be at about 10½ days, is implanted in the endometrium, but has not yet produced an elevation

towards the uterine lumen (Heuser 1940). The trophoblast has differentiated into a cellular and a syncytial layer, in the latter of which are lacunae containing maternal blood. The inner cell mass has differentiated into ectoderm and endoderm and the amniotic cavity has appeared. The abundant growth of early mesoblast is a striking feature and forms an irregular network within the chorion, but there is as yet no evidence of the primary yolk sac.

Insectivora and Chiroptera

The preceding description of implantation does not deal with all modifications found in the different mammalian families. The types described were chosen because they either illustrate a particular method of implantation or placentation, (e.g. epithelio-chorial as in the pig or haemochorial as in man), or because they have been more intensively studied than some other types. Brief mention must now be made of implantation in two important orders, the insectivores and the Chiroptera.

Implantation in the former has been studied by Hubrecht (1889) in the hedgehog, and by Hubrecht (1893) and Brambell and Perry (1945) in the shrew; by Strahl (1892) and Vernhout (1895) in the mole; by Bluntschli (1937) and Goetz (1937 and 1938) in *Centetes*; by van der Horst and Gillman (1942) in *Elephantulus*. In Chiroptera, implantation has been investigated by van Beneden (1899), Duval (1895 and 1896), van der Stricht (1899 and 1909) and Wimsatt (1944) in *Vespertilionidae*; by Sansom (1932) in *Mollosidae*, and by Hamlett (1935) in *Phyllostomidae*.

In insectivores implantation may be superficial as in *Tupaja javanica*, partially interstitial as in the mole and shrew (Fig. 14. 51), or completely interstitial as in the hedgehog (Fig. 14. 52). Implantation occurs in the antimesometrial side of the uterine horn with the inner cell mass orientated towards the antimesometrial side of the uterus. A complete yolk sac is formed as in ungulates and carnivores, but it is never completely separated from the trophoblast by the mesoderm, so that on the abembryonic side a bilaminar omphalopleure persists until term. The allantois varies in size, being small in the shrew, but large in the mole and in *Tupaja javanica*.

In *Elephantulus* extensive changes occur in the uterus during the progestational phase. Before the embryo reaches its site of implantation, the general oedema of the endometrium is replaced by a condensation of the stromal cells. This is followed by the development of a second oedema in the mesometrial side of the uterus, where the embryo is eventually implanted. The egg can only implant at this site and a chamber is formed in the uterine cavity. The epithelial lining of this chamber is now destroyed and the stromal cells show a decidual reaction.

The details of implantation in the vespertilionid bat, *Myotis lucifugus*, have recently been described by Wimsatt (1944). There are typical progestational changes in the uterus during the cleavage stages. From the time the zygote enters the uterus, there is a gradual thinning of the epithelium immediately in relationship with the trophoblast, so that a "pocket" is formed in the epithelium. During the time the blastocyst is free there is a rapid expansion of the antimesometrial side of the uterus with a displacement of the glands. Implantation occurs with few exceptions in the middle of the right horn, which is more developed than the left. At the

time of implantation, the blastocyst comes to lie in the antimesometrial side of the uterus, with the inner cell mass orientated antimesometrially. Unlike the

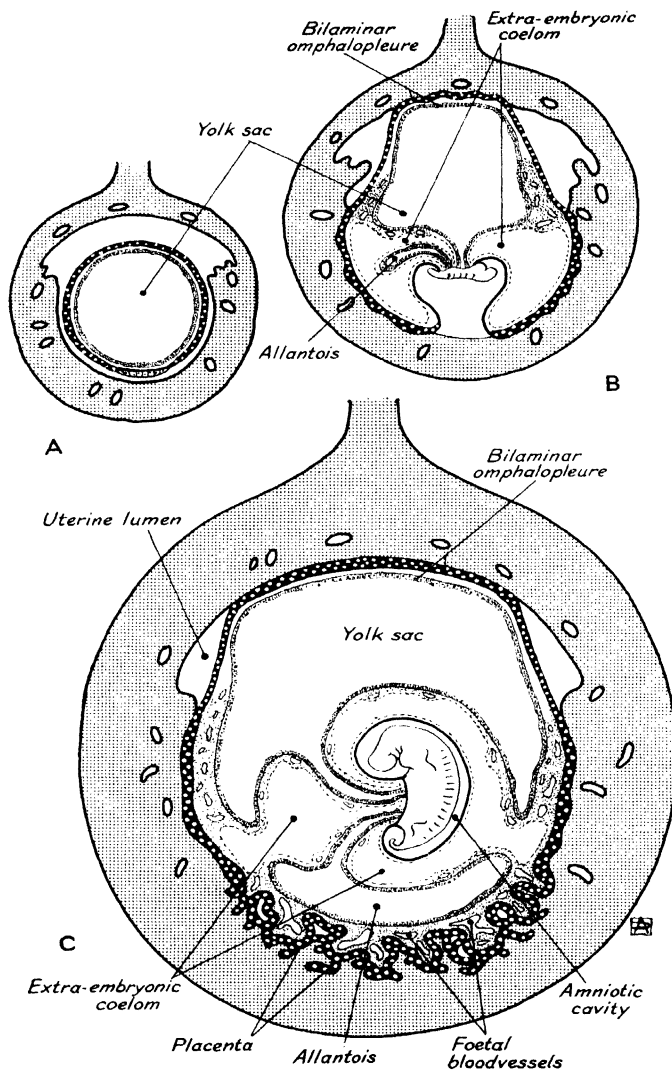


Fig. 14. 51—Schematic transverse sections to show the implantation and placentation of the mole, *Talpa europaea*. Note the absence of decidua capsularis and the attachment of the bilaminar omphalopleure to the mesometrial side of the uterine lumen.

rodent, but like the monkey, the trophoblast over the inner cell mass first attacks the uterine epithelium, which is later destroyed. The blastocyst penetrates into the endometrium but never becomes completely interstitial. Many of the degenerating

cells are incorporated in the trophoblast. In the phyllostomid bat (*Glossophaga*) the implantation is completely interstitial. The trophoblast over the inner cell

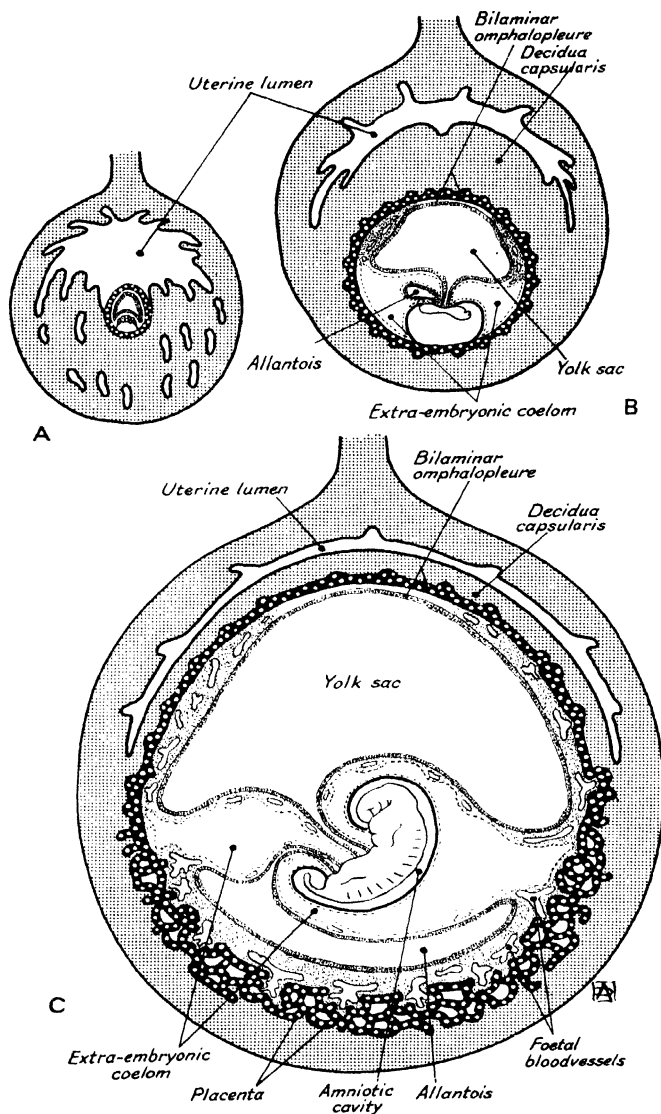


Fig. 14. 52—Schematic transverse section to show the implantation and placentation of the hedgehog, *Erinaceus europaeus*. The implantation here is interstitial and so a decidua capsularis is developed.

mass is thick and actively proliferating as it invades the endometrium. The trophoblast of the remainder, while thick, shows no special proliferation.

IX. EMBRYONIC, OR FOETAL, MEMBRANES

Any structure, or tissue, developed from the zygote which does not form a part, or a significant part, of the embryo itself and is of functional importance only in embryonic life, is called an (extra-) embryonic, or foetal, membrane. These membranes are usually entirely shed or absorbed at birth or hatching. This definition includes embryonic appendages (e.g. the placenta and umbilical cord) which, frequently, are not membranous in character. In the stricter sense of the term, the embryonic membranes found in vertebrates are the yolk sac, the allantois, the amnion and the chorion, but all these membranes are only found in the higher types. Further, in the mammals, a precociously developed membrane, the trophoblast, appears and contributes, in later development, to the chorion and, in many types, to the amnion (*see* p. 98). Its phylogenetic origin and significance require special consideration.

Avian Embryonic Membranes

The condition of the embryonic membranes in the chick embryo may be taken as the convenient prototype (Fig. 14. 53, *see* Lillie, 1927, for details). In the chick the yolk sac, the amnion and the chorion arise from the extra-embryonic blastoderm (*see* p. 55) whereas the allantois has its origin as an outgrowth of the ventral portion of the hindgut. The first membrane to appear is the yolk sac. It is formed by the extension of the endoderm and later of the mesoderm round the yolk and under the peripheral blastodermic ectoderm. When the extra-embryonic coelom appears it splits the peripheral mesoderm into an outer (somatopleuric) layer and an inner (splanchnopleuric) layer. The latter is in contact with the endoderm and together with it constitutes the wall (splanchnopleure) of the yolk sac. The somatopleure is formed by the ectoderm and the somatopleuric mesoderm. With axial growth of the embryo and the formation of its head and tail folds, pockets of the splanchnopleure are drawn into the cephalic and caudal parts of the embryo to form the fore- and hind-guts. The intervening region of the dorsal, embryonic, part of the yolk-sac cavity is the primitive midgut. For some time it remains in free communication ventrally with the yolk-sac cavity. As development proceeds, however, the extent of the ventral opening is progressively decreased until finally it is connected to the yolk sac only by a narrow yolk stalk (vitelline duct). The yolk does not pass directly through this stalk into the embryonic gut, but is "digested" and absorbed by glandular cells in the yolk-sac wall and then passed into the vitelline circulation whose vessels have differentiated in the yolk-sac mesoderm. The amount of yolk is thus progressively reduced and towards the end of incubation the atrophic yolk sac is withdrawn into the embryonic body.

The chorion (or serosa) and the amnion first appear as a fold of the somatopleure near the cephalic extremity of the embryo. At first this somatopleuric fold (the proamnion) is composed solely by ectoderm and endoderm since the mesoderm and the coelom have not yet extended so far forwards. Later, lateral folds and a caudal fold of comparable structure are formed around the sides and caudal extremity of the embryo. Mesoderm is present in these folds and by this stage of development has extended into the proamniotic region. The amniotic folds are formed by the somatopleure. Fusion of the folds occurs gradually over the dorsal aspect

of the embryo, until, at about the 31 somite stage, the amniotic cavity is completely closed. A sero-amniotic connection remains at the position of final closure; elsewhere the separation of an inner layer of somatopleure, the amnion, from an outer layer of somatopleure, the chorion, is complete.

The allantois arises as a diverticulum from the embryonic hind-gut. Its wall is composed of endoderm covered by splanchnopleuric mesoderm. By the fourth day of incubation it extends from the ventral surface of the caudal end of the embryo into the extra-embryonic coelom. The proximal portion persists as a narrow duct, the allantoic stalk, which remains in free communication with the cloacal region of the hindgut throughout incubation. The distal extremity becomes dilated and enlarges rapidly in the extra-embryonic coelom as a flattened sac, spreading round the embryo and the closed amnion, immediately beneath the chorion to the inner surface of which its mesoderm becomes fused. Eventually, together with a duplication of the chorion, it winds itself around the albumen which is now aggregated into a viscid mass. The allantoic mesoderm is very vascular and serves as an embryonic respiratory organ. Its cavity (allantoic bladder) receives the embryo's urine and its wall helps in the absorption of the albumen.

The umbilicus.—The space bounded by the junction of the amnion with the body wall is the umbilicus. The closure of the body wall of the embryo reduces, progressively,

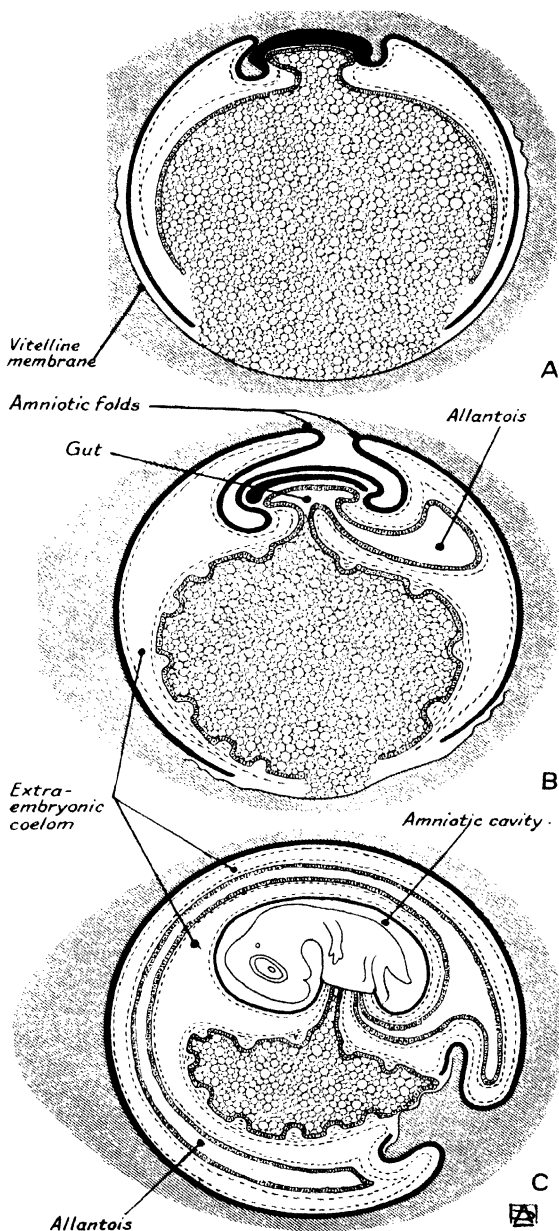


Fig. 14. 53—Three stages in the development of the embryonic membranes of the chick.

the relative size of the opening. *Pari passu* the communication between the embryonic and extra-embryonic coelomata is reduced to a narrow interval between the vitelline and allantoic ducts, on the one hand, and the attachment of the amnion to the body wall on the other.

Embryonic Membranes in Vertebrates

The most primitive of these membranes is the yolk sac. The serosa is a primarily ectodermal membrane which, in the higher types, is usually called the chorion. Part of it is concerned in the development of the amnion, *see* Fisk and Tribe (1949) for details. The amnion is found only in reptiles, birds and mammals, which are consequently classified as *Amniota*. All lower vertebrates are *Anamniota*. The trophoblast is a special mammalian adaptation to viviparity.

Evolutionary origin of embryonic membranes of higher vertebrates

It is generally accepted that the amnion first originated as a fold of blastoderm round the embryonic body. It arises in this fashion in the ontogeny of reptiles, birds and some mammals (*see later*). The blastodermic fold consisted initially of the yolk-sac wall, "the body of the embryo being forced down into the yolk sac as it decreased in size, possibly by the resistance of the rigid protective shell associated with the assumption of a terrestrial habit" (Kerr, 1919). The richly vascularised yolk-sac wall was the embryonic respiratory organ at this stage in amniote development. Increase in its size would cause it to bulge, as amniotic folds, over the body of the embryo and so eventually to utilise all of the inner shell surface. As the egg was now terrestrial the end products of nitrogenous metabolism could no longer pass away by diffusion into surrounding water and the allantoic bladder of amniotes developed precociously as the allantois was able to receive the embryonic urine. The wall of this allantois, being formed of splanchnopleure, was well vascularised and, when it came into contact with the somatopleure, could take part in respiratory exchanges. Growth of the embryo would increase its respiratory demands, and of its two respiratory organs one—the yolk sac—would be decreasing in size while the other—the allantois—would be expanding. This resulted in the supplanting of the yolk sac by the allantois as the main respiratory organ. Development of the allantois and coelom resulted in separation of the splanchnopleure from the somatopleure and the amniotic folds now became purely somatopleural. As soon as the amniotic folds extended to form a complete covering for the embryo "it would at once assume a new importance in protecting the delicate body of the embryo, enclosed within it as in a water jacket, from the dangerous jars and shocks incidental to a terrestrial existence" (Kerr, 1919), *see also* p. 101. It is usually assumed that the development of the amniotic type of egg took place subsequent to the assumption of a terrestrial existence by the early reptiles and that such a development was the first step in the emancipation of these reptiles from the water. Romer (1946), however, has shown that the oldest and most primitive pelycosaurs probably were aquatic and he considers it likely that this represented a retention of the ancestral amphibian mode of life. He makes the further intriguing suggestion that the reptilian egg may have preceded the adult in its adaptation to purely terrestrial conditions. The

mammalian ovum, which is already relatively reduced in size in the monotremes, is very small in the Metatheria and Eutheria. Such an egg possesses neither the food material nor the cubic capacity necessary for the production of an embryo on the ancestral reptilian lines. "We accordingly find that the primary object of the first developmental processes in the mammals has come to be the formation of a vesicle with a complete cellular wall, capable of absorbing nutrient fluid from the maternal uterus and of growing rapidly so as to provide the space necessary for embryonal differentiation" (Hill, 1910). The wall of the blastocyst is the trophoblast and although there is disagreement on the actual homologies of this mammalian embryonic membrane (*see e.g.* Hubrecht, 1908; and McCrady, 1938 and 1944; and van der Horst, 1946) it can best be regarded as precociously developed extra-embryonic ectoderm.

Yolk sac

Amongst Anamniota a definite yolk sac is found only in those which possess megalecithal ova. In such types during, or shortly after, gastrulation all three germ layers extend down over the yolk mass and enclose it within the primitive gut and body wall which, round the yolk mass, constitute a trilaminar yolk sac. The mesoderm of this yolk sac is soon vascularised, and the nutritive material absorbed by the endodermal layer of the yolk sac is transported to the embryo by the vitelline vessels. The yolk sac shrinks as the embryo grows until it is eventually incorporated into the ventral abdominal wall and gut. Its tissues are not merely homologues of the gut and body walls for they actually become parts of these structures. Some sharks and rays are viviparous (Chap. 15) and in them the vascularized yolk-sac wall forms a type of yolk-sac placenta.

In reptiles, birds and monotremes the yolk sac is of the type already described sac for the chick embryo.

In marsupials in spite of the great reduction in the amount of yolk in the egg a yolk sac is developed. After the formation of the endoderm (p. 56) this embryonic layer extends gradually by its own growth round the inner surface of the unilaminar ectoderm so as eventually to form a complete endodermal lining for the blastocyst wall which thus becomes bilaminar. Hill (1910) from his work on the development of *Dasyurus* concluded that the bilaminar blastocyst consists of embryonal and extra-embryonal regions. The former is constituted by an outer layer of embryonic ectoderm with an underlying portion of endoderm (Fig. 14. 56); these two layers will form the future embryo. The extra-embryonal region, separated from the other area by a junctional line, is formed by the trophoblastic ectoderm (tropho-ectoderm) together with the underlying portion of endoderm. The two layers, ectodermal and endodermal, of the extra-embryonal region constitute a bilaminar omphalopleure (or yolk sac). Later in development extension of mesoderm beyond the margin of the embryonal area forms a trilaminar omphalopleure. This mesodermal extension, however, does not, in *Didelphys*, reach more than a third of the distance to the abembryonic pole of the blastocyst (or chorionic sac) and in the abembryonic hemisphere the bilaminar omphalopleure persists throughout gestation (*see* Flynn, 1923, and McCrady, 1938, for details). Formation of the extra-embryonic coelom in the mesoderm of the trilaminar omphalopleure adjacent to the embryonic area results in the separation of this portion of the omphalopleure into an outer (serosal) chorion and an inner (splanchnic) yolk sac. It is into this

restricted coelomic cavity that the small and reduced allantois of marsupials grows. Since the splitting of the extra-embryonic mesoderm into somatopleuric and splanchnopleuric layers only occurs in a restricted region round the embryonic area the definitive chorion in the opossum shows three different parts :— (1) the abembryonic non-vascular yolk-sac chorion (bilaminar omphalopleure of ectoderm and endoderm) ; (2) the vascular yolk-sac chorion (trilaminar omphalopleure) which forms a broad equatorial band round the chorionic sac and constitutes an important organ of nutrition and respiration (yolk-sac or chorio-vitelline placenta, Chap. 15) ; and, (3) the serosal chorion, formed of ectoderm and somatopleuric mesoderm, and concerned in part in the development of the amnion. It will be

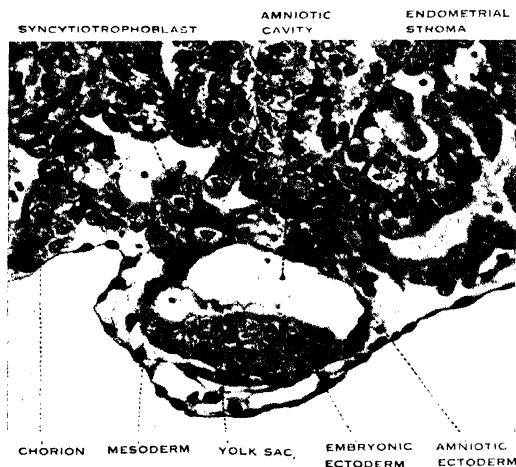


Fig. 14. 54—A section of a 12-day macaque blastocyst. The yolk sac has now made its appearance between the mesoderm and the embryonic ectoderm. $\times 210$. (From Hamilton, Boyd and Mossman, 1945. After Heuser and Streeter, 1941.)

throughout foetal life, but more frequently, as in Primates, it shrivels and disappears.

In human development, and presumably in that of the anthropoid apes, the yolk sac shows certain peculiarities due, it would seem, to the precocious development of the extra-embryonic mesoderm. This mesoderm appears to arise from the inner surface of the trophoblast and differentiates before the endoderm has had time to spread round the interior of the blastocyst. As a result of this the primitive or "primary" yolk sac (Hamilton *et al.*, 1945) has endoderm on its roof only ; the remainder of its periphery is formed by the inner limiting layer (Heuser's or exocoelomic membrane) of the extra-embryonic mesoderm (Fig. 14. 45). Whether this "mesoderm" is truly mesodermal is still in dispute, as it may be formed by mesothelial-like endodermal cells. Later in development the primary yolk sac becomes converted into a "secondary" yolk sac which is relatively smaller and appears to have decreased in size partly as a result of the increase in size of the extra-embryonic coelom and partly by a separation off of its

seen, from this account, that the endoderm of the marsupial yolk sac remains in permanent contact with the outer wall of the chorionic vesicle over most of its extent.

In eutherian mammals the yolk sac is extremely variable. In many species a primitive bilaminar yolk sac (bilaminar omphalopleure) is developed and this may persist in part throughout gestation as, e.g., in the *Sciuridae*. In other Eutheria, by the growth of mesoderm, the bilaminar yolk sac becomes in part, at least, a trilaminar omphalopleure. Vascularisation of this mesoderm may result in the formation of a chorio-vitelline placenta (in certain marsupials, and temporarily in many carnivores, insectivores and rodents). In yet other mammalian types a completely splanchnopleuric yolk sac is developed. This may persist

abembryonic portion (Fig. 14.47). Still later when the fore- and hind-gut diverticulae have been included with the embryonic body that part of the secondary yolk sac which projects into the extra-embryonic coelom may be called "tertiary" or definitive. It is covered by visceral or splanchnopleuric mesoderm and is, of course, completely emancipated from the chorion by the extensive extra-embryonic coelom. Isolated areas of blood cell and blood vessel formation ("blood islands") arise in this mesoderm in close relation to the endodermal cells. These islands extend and become confluent to form an extensive vascular network in the yolk-sac wall. This network soon communicates with vessels within the embryonic body by way of paired vitelline veins and arteries. The functional significance of the extra-embryonic vitelline circulation is obscure. It is obviously important in haematopoiesis and it may also be concerned in absorption (Streeter, 1942).

In the macaque monkey (Heuser and Streeter, 1941) the primary yolk sac, essentially like that of other mammals, does not give rise directly to the secondary yolk sac. The latter seems to have its origin in a rearrangement of the originally flat plate of embryonic disc endoderm which forms a small separate cavity surrounded by haematopoietic mesoderm. The cavity of this "secondary" yolk sac has no obvious continuity with that of the "primary" sac. Hamilton *et al.* (1945) have suggested that the relatively direct and early method of separation of these two parts of the yolk sac in *Macacus rhesus* is the result of an abbreviated and specialised method of "pinching off" of its distal abembryonic portion (Fig. 14.54).

In many rodents the embryonic ectoderm becomes invaginated into the embryonic portion of the yolk sac so that the latter is "inverted" (inversion or entypy of the germ layers, Fig. 14.38). Later the peripheral, or distal, part of the yolk-sac wall disappears so that its cavity is opened up. In this manner the inner, or proximal, part of the endoderm is brought into contact with, or close proximity to, the uterine tissues, and the former yolk-sac cavity may become confluent with the uterine lumen. The functional significance of these curious processes is unknown, but the arrangement persists until term and is probably of physiological significance. Some degree of entypy is also found in the development of many bats, insectivores and in the dasypodine armadillos. (For further details on entypy see Mossman, 1937, who summarises the earlier literature; Everett, 1935; Nielson, 1940; Snell, 1941; and Brambell and Perry, 1945.)

Chorion and trophoblast

The term chorion is used loosely to denote a membrane enclosing the developing ovum, and is even sometimes applied to an egg membrane. In vertebrate embryology, however, it is usually applied in a more restricted sense to the extra-embryonic somatopleure, exclusive of that part of it that forms the amnion (*see p. 98*). It is composed of extra-embryonic ectoderm and the somatopleuric extra-embryonic mesoderm. In the Sauropsida and the Monotremata it is frequently called the serosa. In these vertebrates the chorion appears concomitantly with the amnion and the two layers are usually joined throughout incubation by a persistent chorio-(sero-)amniotic connection at the point of final closure of the amniotic cavity. The sauropsidan chorion forms a covering for the embryo and the other

membranes, and a lining for the shell. The splanchnopleuric mesoderm of the allantois becomes applied to the mesoderm on the inner surface of the chorion and it is through these layers that the respiratory exchanges take place.

In marsupials and Eutheria the chorion is derived from the whole or part of the wall of the blastocyst. This wall was called the trophoblast by Hubrecht. It is usually considered to be ectodermal in nature, though, as McCrady (1944) has pointed out, in some types (e.g. the marsupials where no inner cell mass is present) it would be preferable to consider it as a layer having greater potentialities than producing ectodermal derivatives alone. McCrady, in fact, has suggested that it should be called the protoderm, and Hertig (1935) showed that in *Macacus rhesus* it gives direct origin to extra-embryonic mesoderm. Whatever its phylogenetic origin and nature may be, it is the trophoblast which forms the outer, bounding, layer of the developing mammalian zygote and through it in the early post-cleavage stages of development all exchanges between embryo and maternal organism must take place.¹ In many eutheria, indeed, the trophoblastic bounding layer persists throughout gestation.

The trophoblast in the course of development becomes lined with extra-embryonic somatopleuric mesoderm and the two layers together are called the chorion ("true" chorion or serosa). In many types, further mesoderm is brought to the trophoblast by the expansion of the allantois (p. 101) and the vascularisation of the inner layer of the chorion is derived from this splanchnopleuric mesoderm. The fusion of allantois with chorion (chorio-allantois) is the basis of the most important type of placenta (chorio-allantoic, *see* Chap. 15) found in mammals. In many types, however, either as a transitory phase in development or throughout gestation, there is a fusion of the yolk sac with the trophoblast or the chorion. If the fusion is of endoderm with trophoblast, the result is a bilaminar omphalopleure; if there is interposed mesoderm it is a trilaminar omphalopleure. When the interposed mesoderm is vascularised a chorio-vitelline placenta is produced.

In addition to the many differences that can be established between the nature of the mesoderm lining the chorion and its vascularisation in the different mammals, there are marked differences in its size and shape and thickness. In the ungulates, for example, it grows at a prodigious rate between the 12th and 15th days of development (Figs. 14. 29 and 30) to become a very large, thin-walled sac. In mammalian embryos with eccentric or interstitial implantation, however, the chorionic vesicle grows much more slowly and its wall, especially its trophoblastic component, tends to be much thicker.

There are also striking variations in the differentiation of the trophoblastic portion of the chorion in different mammals. These differences can also be correlated, in a general way, with the nature of the implantation and the type of placentation. Further, as would be expected, different parts of the chorionic wall show different structure, depending on the placentation and the stage of development.

In human development, as in most mammals (Figs 14, 44 and 47) the trophoblast becomes differentiated from the inner cell mass by the appearance of the

¹ In some mammals, however, the trophoblast (Raubert's layer) over the inner cell mass disappears and the amnion is formed by folds (p. 98, and Fig. 14. 56). In others the abembryonic trophoblast disappears completely in later development and the yolk-sac wall or the yolk-sac cavity are exposed (Figs. 14. 37 and 38).

blastocyst (blastocoelic cavity). The trophoblast is thus the first embryonic membrane to arise and initially it has the form of a unilaminar membrane. With the

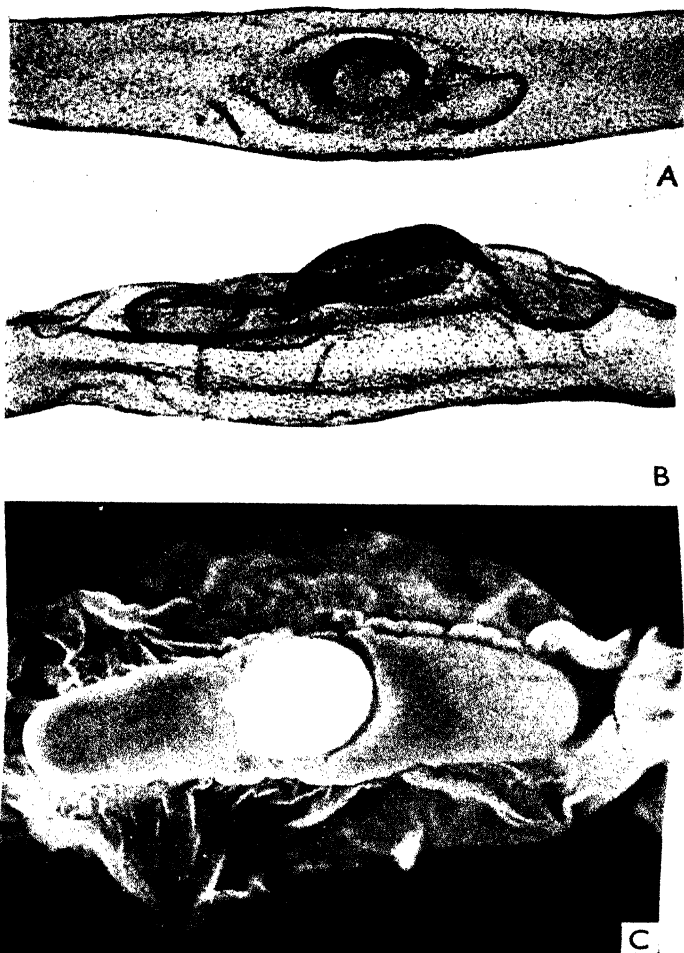


Fig. 14.55—A. Embryonic disc in 13-day sheep embryo. B. Embryonic disc region in a 14-day sheep embryo. C. Surface view of embryonic disc of a 14-day sheep embryo. The extra-embryonic coelom appears as a sausage-shaped cavity arranged circumferentially around and partially underlying the circular embryonic disc.

development of the extra-embryonic mesoderm the trophoblast becomes the chorion. This membrane, with the amnion and primary yolk sac represent a segregation of the materials for membrane formation from that material out of which the body of the embryo will be formed. The changes in the human trophoblast

that occur during implantation and the formation of the early placenta have been described on p. 80. Later changes are considered in Chap. 15.

Amnion

As has been stated earlier, this membrane is found only in reptiles, birds and mammals. In phylogeny it was primarily a modification of the extra-embryonic body wall (somatopleure) which formed (amniotic) folds over the embryo. By fusion of these folds the embryo came to be enclosed completely within a liquid-filled space—the amniotic cavity. The amnion still arises by fold formation in the amniotes (reptiles, birds and monotremes) with megalecithal eggs and, also, in the marsupials and many Eutheria. It is in this manner, for example, that the amnion arises in the ungulates (Figs. 14. 28, 55 and 56). Amnion formation by folds has been called *plectamnios* (da Costa, 1920) and this method of amniogenesis is generally considered to be primitive in mammals. In many mammals, however, the amniotic cavity arises as a space, or a confluence of spaces, in the substance of the inner cell mass of the blastocyst (Fig. 14. 56). This method of formation of the amniotic cavity was named *schizamnios* by da Costa. In some of the mammals showing this method of amnion formation, however, the trophoblast overlying the initially closed amniotic cavity disappears and a secondary amniotic cavity is later formed by fold formation (Fig. 14. 56). In other mammals the initial cavity (ecto-trophoblastic) is situated between the embryonic ectoderm of the inner cell mass and the trophoblast; here, again, the definitive amniotic cavity is formed secondarily by folds of ectoderm, deep to the intact trophoblast (Fig. 14. 56). The trophoblast overlying the inner cell mass has a very variable history in the different mammalian groups—frequently becoming a very thin membrane, known as Rauber's layer, and often disappearing completely (Figs. 14. 28 and 56). In the rodents with marked entypy of the germ layers, a cylindrical column of ectoderm extends into the inverted yolk sac from the trophoblast. This column is called the *träger* or *suspensorium*, and in it a space (epamnionic or pro-amnionic) appears (Fig. 14. 37). In the course of development this space becomes divided by the formation of amniotic folds (or projections, Figs 14. 37 and 56) into a lower (embryonic) cavity, which is that of the definite amnion, and an upper (trophoblastic) ectoplacental cavity. The latter is transitory and is eventually completely obliterated by the expansion of the extra-embryonic coelom and the elaboration of the trophoblastic portion of the placenta (Figs. 14. 37).

There has been much discussion on the phylogenetic significance of these different methods of amnion formation (Hubrecht, 1895; da Costa, 1920; Hill and Tribe, 1924; Mossman, 1937). The usually accepted interpretation is that amnion formation by folds is the primitive mammalian method and that amnion formation by cavitation, in its various forms, is an adaptation to the early time of implantation, or the nature of implantation, in those mammals possessing a schizamnion. Mossman (1937) has demonstrated the existence of transitional types in rodents, e.g. in *Geomys* and *Dipodomys*. In *Geomys*, entypy occurs soon after implantation and the amniotic folds arise at this relatively early stage and fuse to form a closed true amniotic cavity and an open trophoblastic (or false amniotic) space. *Dipodomys* implants somewhat earlier and also shows inversion earlier. The early "amniotic tube" so formed, remains open temporarily, but later its sides fuse to form a small, true amniotic and a much smaller closed tropho-

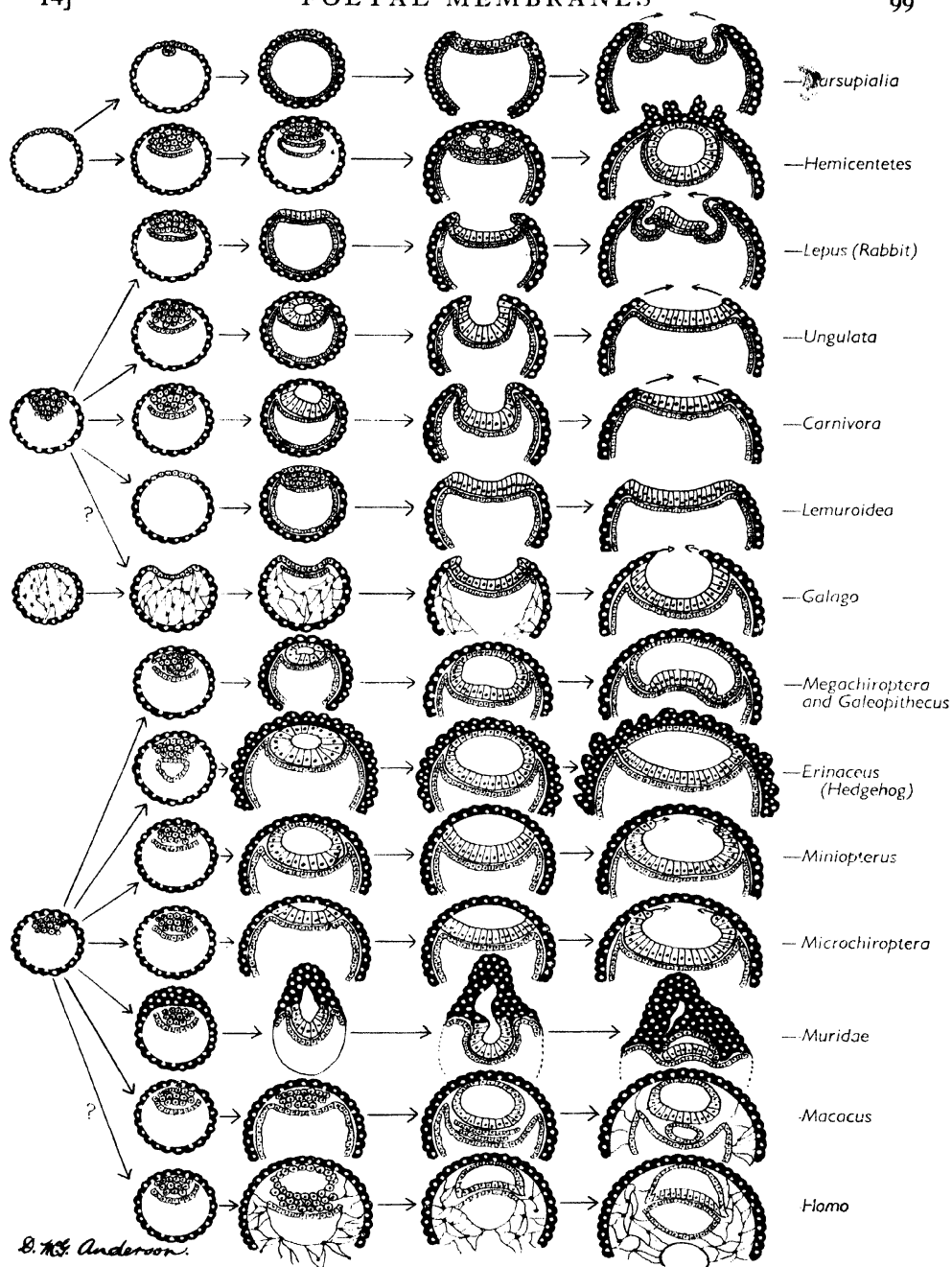


Fig. 14. 56—Schematic representations of formation of the amnion, yolk sac, trophoblast and embryonic disc in different mammals, to show the variations which have been described up to the present. Based in part on Goetz, 1938.

blastic cavity. The next step appears to be that found in the Muridae (Figs. 14. 37 and 56), where the inner cell mass shows two, sometimes three, regions of cavitation. These cavities coalesce, but are soon separated by the amniotic folds. Finally in the guinea-pig, the lowermost (amnio-embryonic) portion of the inner cell mass separates from the Träger before cavitation occurs in either (Fig. 14. 56); that is, the two cavities are formed and separate long before there is any mesoderm formation and there are no amniotic folds.

Mossman has pointed out that it can be seen from such a series, that the type of amnion formation is correlated with the method of implantation. In rodents with superficial or only partially interstitial implantation (e.g. *Lepus* and the Sciuridae), the following method is unmodified. In *Geomys*, with intramural implantation of a large blastocyst and where the uterine epithelium remains open over the site of penetration, there are definite amniotic folds, though they close early and result in the formation of a true amnion, and an open trophoblastic cavity. In *Dipodomys* and the Muridae, the amniotic folds are very much reduced and in *Cavia*, no obvious relation to the folding method can be seen. In other mammalian orders (Insectivora, Chiroptera and Primates) similar, though not so clear, variations are found and the same principle appears to hold, viz., that in superficial implantation amniogenesis by folds is constant, but in the interstitially implanting types, or those with early attachment in the embryonic disc region, it tends to be by cavitation.

In the development of *Macacus rhesus*, Heuser and Streeter (1941) have suggested that the germ disc portion of the inner cell mass comprises the pluripotential residuum of the cytoplasm of the original fertilised egg, freed of the auxiliary tissues. They consider that the amnion is quite different, being derived, by means of amniogenic cells, from the adjacent trophoblast. These cells unite as a membrane over the germ disc and are continuous with its peripheral margins. Later the amnion closes in snugly over the growing embryo and its line of continuity with the embryonic ectoderm narrows down to the umbilical ring. Finally, the amnion becomes filled with fluid and by its expansion comes to obliterate the large extra-embryonic coelom except for a thin reticular stratum that persists between the outer surface of the amniotic membrane and the chorionic wall. In some of their specimens, Heuser and Streeter observed detritus and blood cells of maternal origin in the amniotic cavity. They regard this as abnormal, but this appearance can also be found in early embryos of the bat, *Miniopterus dasythrix* (personal observations), and remains a matter for explanation.

In its later history, Mossman (1937) has shown that the amnion exhibits three general types: (1) it may remain an independent non-vascular membrane until full term, as in most animals with a moderately small allantois (Sciuridae) or no allantoic vesicle (Geomysidae); (2) it may expand to such an extent as to obliterate almost all of the extra-embryonic coelom, its mesodermal covering fusing with the chorion or chorio-allantois, as in man; (3) it may become surrounded by the allantois; fusing with the adjacent wall of the latter and thus becoming vascularised, as in the Artiodactyla, Perissodactyla and Carnivora. Mossman points out that the explanation of these three different types seems to be related more to the condition of the allantois than to any physiological adaptations of the amnion itself, for the functions of the latter seem to be equally well served under any of the three arrangements.

On the function of the amnion, Mossman has stressed that it should primarily be considered an adaptation to provide embryos of non-aquatic vertebrates with an aquatic environment. "It offers practically no 'protection' against mechanical shock or desiccation; in fact, the amnion itself needs protection against just these things to insure its proper function. The shell, shell membranes, albumen or maternal tissues furnish this."

Our knowledge of the sources and control of production and absorption of the amniotic fluid is scanty (*see* Needham, 1931; and Windle, 1940, for discussions). In human material the amniotic sac is known to fill with fluid and to expand at the expense of the extra-embryonic coelom, even in those instances where, owing to abnormal development, the embryo remains a mere rudiment. Such findings demonstrate that the amniotic fluid is not solely a product of the embryo itself. The fluid was considered to be relatively stagnant, until Flexner and Gellhorn (1942) demonstrated, by the use of isotopes in the guinea-pig, that the aqueous content of the fluid is replaced about once an hour, while the rate of replacement of sodium is about 50 times slower. Recently Vosburgh *et al.* (1947) have measured water and sodium transfer to the human amniotic fluid using deuterium oxide and radioactive sodium as tracer substances. They have demonstrated, on a series of cases, that on the average 0.345 ml. of water is transferred to a ml. of amniotic fluid per hour; i.e. the water of human amniotic fluid is "turned over" once every 2.9 hours. The sodium is "turned over" once every 20.5 hours. Such results render it evident that the membranes, traversed by substances reaching the amniotic fluid from the maternal circulation, are far more permeable to water than to sodium and that the water is replaced with astonishing rapidity.

In structure the amnion of mammals consists of an inner layer of flattened ectodermal cells surrounded by a thin layer of mesoderm. In birds and reptiles unstriped muscle cells are differentiated in this mesoderm. In the chick embryo the muscle fibres first appear on the fifth or sixth day of incubation and gradually increase in number. They disappear later in the region of fusion of the amnion with the allantois, but persist elsewhere. By their vermicular contractions they are believed to prevent adhesions of the amnion to the embryonic ectoderm and it is possible that they are active in the inclusion of the yolk sac within the embryo. The amnion is usually non-vascular, but is secondarily vascularised in ungulates, and Noback (1946) has described vasculogenesis in the amniotic mesoderm coat in an embryo of the baboon, *P. papio*. Mossman and Noer (1947) have reported briefly on the structure of the amnion of the mouse as revealed by the electron microscope.

Allantois

The phylogeny of the allantois has already been discussed and it has been pointed out, that this membrane arose as a diverticulum of the ventral (urogenital) portion of the cloaca. Its origin and developmental history in the chick embryo are briefly described on p. 91. A closely comparable development is shown by other birds and by reptiles. The phylogenetic relationship of the allantois to the anamniotic bladder is still uncertain, but the two structures are usually considered to be homologous (for discussion *see* van den Broek *et al.*, 1938). Whatever its phylogenetic history, however, the sauropsidan allantois is always formed by

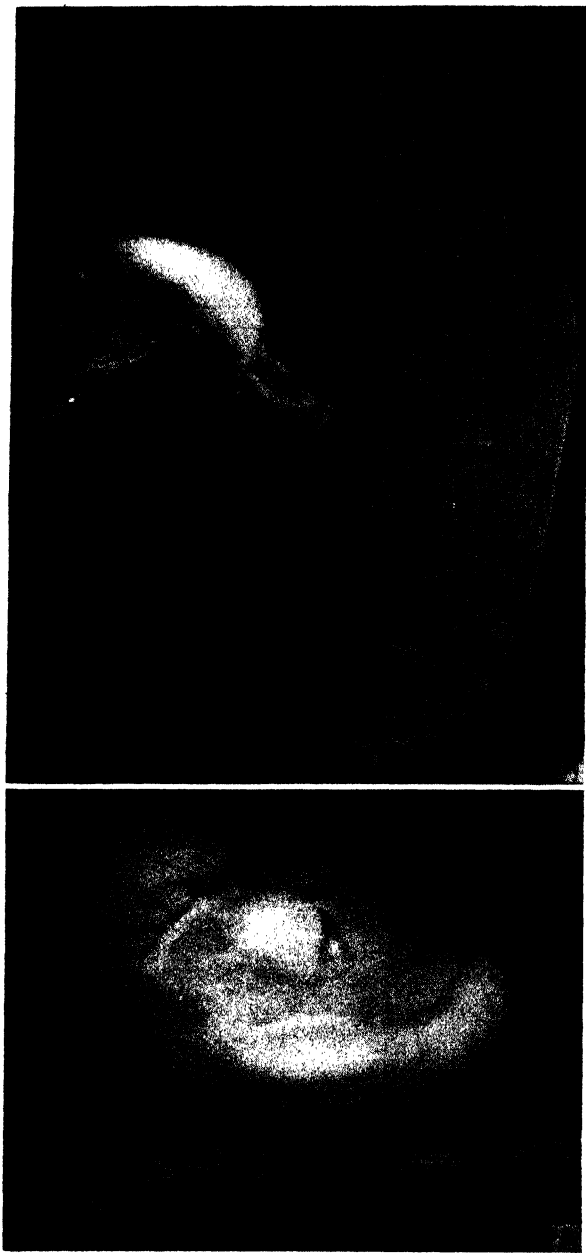


Fig 14. 57.—A. An 18-day sheep embryo to show the relatively enormous size of the allantois. B. A 19-day sheep embryo to show early vascularisation of the allantoic diverticulum. $\times 15$.

the ventral endodermal wall of the hind-gut and is covered by a vascular layer of splanchnopleuric mesoderm.

In Mammalia, the allantois is very variable in the extent of its development and in function. In the monotremes, it is similar to that of Sauropsida. In the marsupials, it is usually small and in most of them is situated in an invaginated pocket of the yolk sac so that it does not reach the surface of the chorion. Hence, a choro-allantoic placenta (see Ch. 15) cannot be established, (see McCrady, 1938, for details in *Didelphys*). In *Perameles*, however, Hill (1897), and Flynn (1922 and 1923) have shown that the allantois does reach the chorion and is concerned in the establishment of a placenta of the chorio-allantoic variety. A similar relationship is present in certain viviparous lizards (see Ch. 15).

In the eutherian mammals, there is extreme variation in the development and nature of the allantois. Nevertheless, as Mossman (1937) has indicated, there is a fairly constant condition and size of the allantois within each mammalian order. Thus, a large endodermal allantoic vesicle is found in the Carnivora, Cetacea, Tubulidentata, Proboscidea, Sirenia, Lemuroidea, Perissodactyla, Artiodactyla and Hyracoidea. In these Eutheria, the large allantois frequently possesses a bizarre shape and its cavity shows loculations. In the sheep, for example, soon after its origin the allan-

tois becomes subdivided and appears as a large anchor-like diverticulum of the hind-gut at the caudal end of the embryo (Figs. 14. 31 and 57). The great size of the allantois in the embryo of these mammalian orders may, in part, be accounted for by the great size of their chorionic sacs, and the nature of implantation (p. 64) and placentation (Chap. 15). The loculations, however, do not seem to have any functional significance. In these orders, the allantoic cavity contains large amounts of fluid which fluctuates in volume during gestation. The significance of this fluid is unknown, but part is stored urine. The endodermal allantois in Insectivora, Dermoptera and Chiroptera is of moderate size. In Primates (excluding the Lemuroidea, *see* Hill, 1919, and Hill *et al.*, 1928, for discussion), Tarsioidea, Edentata (except the Manidae) and Rodentia (Fig. 14. 37) the endodermal allantois is small, rudimentary or completely absent. In spite of the vestigial nature of the endodermal allantois in the last group of mammalian orders, however, the allantoic mesoderm is well developed. This mesoderm often develops precociously (Hill, 1932) and forms the stroma of the connecting stalk (*see* p. 77). Indeed, Hill considers that the extra-embryonic (primary) mesoderm of Primates, should be regarded as precocious allantoic mesoderm and this interpretation satisfies many difficulties in the homologisation of early primate development with that of other Eutheria.

A consideration of the details of development of the allantois in mammals and of its blood supply is beyond the ambit of this account (*see* Grosser, 1927, Mossman, 1937, and Needham, 1942). Further details of functional importance are considered in the next chapter. One final point, however, deserves some mention. In Sauropsida and monotremes the allantois functions as a respiratory organ, as a place of storage for the urine and as a mechanism for helping in the absorption of the albumen. In the marsupials, except *Perameles*, the yolk-sac placenta carries out the respiratory and absorptive functions and the allantois is small. In Eutheria, however, the vascularisation of the chorio-allantoic placenta is by the allantoic vessels and the allantoic mesoderm is consequently of great functional importance. Bremer (1916) demonstrated that there is a relationship between the nature of the placental membrane, the size of the allantoic endodermal sac and the degree of development of the mesonephroi. Thus in animals with a large allantois there are a relatively thick placental membrane and very large mesonephros, while in those with small or absent endodermal allantois the placental membrane is thin and the mesonephroi are very small. The correlation, however, is not applicable to all types and, as Gersh (1937) has shown, the fact that the mesonephros and the metanephros can, and probably do, function simultaneously and continuously gives little meaning to Bremer's comparison.

Reichert's Membrane

In addition to the embryonic membranes already discussed there is, in certain mammals, an additional structure which, though non-living, must be considered as an accessory membrane. It is named after its discoverer, Reichert (1862), who found it in the guinea-pig, where as has been explained (p. 95), the yolk sac has peculiar relations (entypy) to the other embryonic membranes. In spite of Snell's (1941) assertion that Reichert's membrane is only found in the Rodentia, it is also present in Insectivora (*Crociodura caerulea*, Sansom, 1937; *C. russula*, personal observations; *Sorex araneus* and *minutus*, Brambell and Perry, 1945).

In the mouse, Reichert's membrane appears during the seventh day of gestation as a thin, homogeneous, non-cellular membrane, which stains pink with eosin, between the trophoblastic ectoderm and the distal yolk sac endoderm in the abembryonic region. It soon becomes continuous over the entire inner surface of the trophoblast (trophectoderm). When the latter disappears the membrane seems to be anchored to the surrounding uterine tissues by the giant trophoblastic cells (see Chap. 15).

There is uncertainty as to the origin of Reichert's membrane, some investigators attributing its formation to the endoderm, others to the trophoblast. The general opinion of those who have worked on the rodents is that the membrane is a product of the endodermal cells. Sansom suggested that in *Crocidura* the trophoblast may be responsible for its growth, but considered that it is laid down, at least in part, by the endoderm of the bilaminar omphalopleure. Brambell and Perry have, however, provided strong evidence for its derivation from the trophoblast in *Sorex*.

The function of Reichert's membrane is not clearly understood. It can obviously act as a limiting membrane to the uterine tissues in the types with marked entypy and disappearance of the trophoblast. Everett (1935), working on embryos of the albino rat, has shown by perfusion experiments that the membrane forms the parietal boundary of the yolk sac and is bathed directly by circulating maternal blood. He suggests that it allows substances to diffuse into the yolk sac directly from the blood and that yolk-sac nutrition in the rat is haemotrophic rather than histiotrophic. Everett demonstrated that Reichert's membrane readily allows the passage of colloidal trypan blue but not of iron ammonium citrate. This finding led him to conclude that the membrane should be regarded as an integral part of the yolk-sac placenta.

X. ABNORMAL DEVELOPMENT AND PRENATAL DEATH OF THE EGG, THE ZYGOTE AND THE EMBRYO IN MAMMALIA

Adult individuals of a given animal species show variations which are the result of genetic differences, and random alterations in the environment during ontogeny. An averaging of these intraspecific variations leads to an abstract conception of a "norm" for that species, to which the majority of its members approximate. Those individuals within close range of the norm have had normal development ("normogenesis"). Those outside this more or less arbitrary range have undergone abnormal development ("teratogenesis") resulting in structural malformation, functional inefficiency or both. Owing to the phenomenon of embryological regulation temporary abnormalities in development may be "compensated" in later stages. Many abnormalities, however, are persistent in their effects; some make postnatal existence impossible, others may cause embryonic or foetal death. In some instances death of the embryo is due to the environmental conditions in which it is placed; in others it is the result of innate deficiencies, e.g. the possession of homozygous lethal genes.

In many mammals the extent of prenatal mortality, from conception to birth, is known to be considerable though there is much variation, even between different races and strains of a given species. In polytocous species litter size is obviously dependent on the extent of embryonic mortality. Litter size, however, is not

convincing evidence of prenatal mortality, for differences between different strains and species in the number of young at birth may be due to differences in the number of eggs per ovulation. As early as 1842 Bischoff drew attention to the fact that, in polytocous mammals, the number of corpora lutea usually corresponds to the number of embryos. In the rabbit, however, especially in the later stages of development, he occasionally found fewer embryos than corpora lutea. He explained this discrepancy by the death and abortion of some of the embryos. Similar discrepancies have been observed and interpreted in the same manner by many subsequent observers (*see* Robinson, 1921, and Corner, 1923, for reviews of the earlier literature).

During incubation in birds and gestation in mammals many factors may prevent the development of the embryo and there are definite critical stages in development when death is more frequent. Thus Payne (1919) has shown that there are usually two peaks in the graph of mortality rate during incubation of hen's eggs, one on the 4th or 5th day, the other on the 17th to 19th day. Needham (1931) speculates that the first peak may be due to the change from allantoic to pulmonary respiration which occurs at this time. The second may be due to malposition of the chick in the egg.

In mammals the period of implantation is a particularly critical one for survival of the blastocyst. Defects in the implantation mechanism or in the supply of (or incorrect synergistic balance between) the different hormones necessary for the maintenance of pregnancy may lead to the absorption or abortion of the embryo. The hormones may act directly on the blastocysts (Corner, 1928; Bloch, 1939; and p. 38) or they may affect the developmental environment by their actions on the uterine tissues, especially the decidua (*see* p. 62). Thus in the cow and sheep Hammond (1941) has suggested that the failure of implantation of eggs ovulated under the influence of gonadotrophic hormones and fertilised by artificial insemination (Zawadowsky *et al.*, 1935a and b) may be due to the absence of receptivity in the uterine mucosa at the time when the blastocysts should become attached. Injections of oestrogen given during early pregnancy will inhibit the actions of progesterone and result in failure of attachment of the blastocyst. If given later they may cause absorption or abortion in rats (Smith, 1926), mice, rabbits (Parkes and Bellerby, 1926; Courrier and Kehl, 1933; Courrier and Raynaud, 1934) and cats (Courrier and Gros, 1935). Death of a mammalian egg or embryo, however, may occur before fertilisation, at fertilisation, during or after attachment to the uterine mucosa. There is an enormous mortality amongst ova in the preovulatory stages (*see* Section on atretic follicles, Chap. 5). The mechanism of selection of the particular ovum, or ova, destined to be shed is still a matter for speculation; the actual number ovulated in a given species is known to be under hormonal control (Chap. 21). Obviously, however, there must be some form of intra-ovarial competition.

After ovulation, death of the ovum is almost inevitable in the absence of fertilisation (*see*, however, parthenogenesis, p. 41). Failure of fertilisation may be due to an inherent or acquired (e.g. age) inability of the ovum to be penetrated by a sperm. In the ferret, for example, Hammond and Walton (1934) found that the ovum remains capable of fertilisation for not more than thirty hours after ovulation. From 18–30 hours after ovulation only a small proportion of the ova can be fertilised and the life of the sperm in the female reproductive tract is relatively short, possibly not more than 30 hours (Hartman, 1945). Lowered fertility and smaller litter

TABLE V

CAUSES OF FOETAL DEATH AND ABNORMALITY (modified from Corner, 1923)

A. *Defects of zygosis*

- | | | |
|---|----------|--|
| 1. Failure of fertilisation | | a. Abnormalities in sperms or seminal fluid |
| 2. Irregularities of fertilisation | } due to | b. Abnormalities in their membranes |
| 3. Delayed fertilisation | | |
| 4. Fertilisation in which only one pronucleus participates in later development (gynogenesis and androgenesis). | | c. Time interval between mating and ovulation in those species where sperms and/or ova have limited lives. |

B. *Defects in zygote*

1. Genetic.
 - a. general.
 - b. local
2. Accidents (e.g. fusion of membranes in twinning resulting in hormonal disturbances—free-martins).
3. Infections (e.g. syphilis in man, *Brucella abortus* in cattle, virus abortion in mares, and certain other virus infections—rubella, morbilli, variola, varicella).

C. *Faulty maternal environment*

1. General. Health, age and nutrition (including vitamins). Whether primigravida or multigravida. Maternal infection and toxæmia. Blood incompatibilities (e.g. rhesus factor).
2. Local.
 - a. Mechanical disorders of uterus (e.g. displacements and abnormalities).
 - b. Infection of reproductive tract (e.g. salpingitis, endometritis, cervicitis, vaginitis).
 - c. Faulty hormonal influences
 - (α) on endometrium (e.g. diminished secretion, absent decidual reaction).
 - (β) on myometrium (e.g. abnormal motility).
 - d. Toxic conditions e.g. lead or silver).

size consequent on late fertilisation has also been demonstrated in the ewe (Quinlan and Maré, 1931), in the rabbit (Hammond, 1934), in the mare (Hammond, 1938), in the mouse (Long and Mark, 1911; Lewis and Wright, 1935), in the sow (Lewis, 1911), and in the guinea-pig (Blandau and Young, 1939). The last named investigators found no normal development following insemination 20 hours after ovulation and complete absence of development after insemination 32 hours after ovulation. The effects were due to the age of the egg at the time of fertilisation rather than to faults in implantation, and the early termination of many pregnancies (before the 27th day) could be attributed to delayed fertilisation. Hammond (1934) has shown that the rabbit egg is incapable of being fertilised after it has acquired a layer of albumen and he demonstrated the importance of the sperm "swarm" in the upper part of the oviduct at the time of ovulation. Walton,

et al. (1940) have summarised the factors which may affect the chances of fertilisation as follows:—(1) the time interval between mating and ovulation; (2) the time period during which the ovum remains fertilisable in the female tract; (3) the number of spermatozoa which reach the uterine tube; (4) the time taken by the sperms to make the ascent; and (5) the time period that the sperms can remain in the uterine tube and still be capable of effecting fertilisation. Polyspermy may be a causal factor in mammalian abnormalities, and abnormal pronuclear stages have been observed in some species (e.g. ferret and hamster). Treatment of the seminal fluid with X-rays may result in absence of, or faulty, development when this fluid is used for artificial insemination (Snell, 1935, and Amoroso and Parkes, 1947, and see discussion on p. 41, parthenogenesis). Corner (1923) has classified abnormalities resulting from faulty or atypical fertilisation as defects of zygosis (see Table V). During cleavage and in blastular stages in some divergent mammalian types there is a high mortality. The elephant shrew (*Elephantulus myurus jamesoni*) is remarkable among the eutherian mammals for the high prenatal death rate of its cleaving eggs (van der Horst and Gillman, 1942). As many as 60 eggs can be discharged from each ovary, yet only one embryo develops in each uterine horn. After ovulation the eggs are gathered into a special dilated part of the fallopian tube where they remain for some time. Here the eggs are fertilised and cleavage begins. Many of the eggs fail to cleave and none develop beyond the four-cell stage unless they reach the implantation site. The elimination of the excess of fertilised eggs is due to the fact that implantation can only occur in a very restricted area of each uterine horn (p. 87). In the egg of *Hemicentetes*, another insectivore, Bluntschli (1937) and Goetz (1938) also found from 23 to 40 early developmental stages in the uterine horns, even though the maximum litter size was only ten.

In the polytocous marsupials there is a high "pre-pouch" death rate. In *Dasyurus*, Hill (1910) found that the number of nipples of the adult female is six (occasionally eight). This number obviously predetermines the maximum number of young that may be reared. As 30 (or even more) ova may be liberated at a given ovulation a high proportion of the newly born young must die. This loss does not account completely for the "pre-pouch" mortality as abnormal cleavage stages are frequently found.

Hartman (1928 and 1929b) has given an account of the number of eggs discharged at one ovulation in the opossum. He found as many as 56 though more than 30 is rare. The average number discharged by the opossum is 22, 11 from each ovary. The fate of the unfertilised uterine egg of the opossum has been given by Smith (1925). That death after implantation does occur was demonstrated by the observations of Hammond (1921) in the pig, Corner (1923) in the pig, Stockard and Papanicolaou (1918) in the guinea-pig, and by Robinson (1921) in the ferret. Hammond found that 15 out of 170 embryos in the uteri of 7 sows were atrophic. His data showed that the atrophy had commenced at different times in different embryos, or had proceeded more rapidly in some embryos than in others. In the rabbit, Hammond found 82 of 307 zygotes to be atrophic. The total number of zygotes present (normal and atrophic) did not correspond to the number of ova shed. In the ferret, Robinson found that some ova were unfertilised, others showed degeneration during the cleavage stages, while still others were degenerating after their attachment to the uterus. He estimated that there is 35 per cent. mortality

in the ferret. Since normally developing ferret zygotes were found in the same tubes as those that were degenerating Robinson concluded that the general state of the oviduct and uterine cornua were not the cause of death of the extruded ova. He considered that much of the prenatal death which occurs in mammals is "normal" and is due to abnormalities in the zygotes themselves.

Robinson was not able to find all the ova shed in the oviduct or uterine horns. He also found that ova may escape through the aperture of the periovarial capsule into the general peritoneal cavity and in one series of cases he found that approximately 15 per cent. of ova were lost in this manner.

Parkes (1923) was the first to study foetal regression in mice. Using indirect methods he found that 10·8 to 23·1 per cent. of ova were not represented by living young at birth. MacDowell (1924) found that of 1,274 corpora lutea (average of 9·4 per oestrus), in mice from normal strains, 842 resulted in living young at birth (average of 6·2 per litter). These figures give a prenatal mortality of 33·9 per cent. Embryos which die before implantation were completely phagocytosed; those dying at later stages were completely resorbed. As the data revealed a decline in litter size from the fourth or fifth litter onwards whereas the ovarian activity (i.e. fecundity) showed no such trend MacDowell concluded that the decrease in litter size in older females was chiefly attributable to an increase in prenatal mortality. In his smaller mice litters, Parkes (1923) found an excess of females, but in the larger litters an excess of males. He, therefore, assumed that the smaller litters had suffered a large prenatal depletion due to excessive death of the males. Bittner's data (1936) support Parkes' findings. MacDowell and Lord (1925 and 1926) found, however, that in the absence of prenatal mortality the sex ratio was unity and that in litters with foetal regression there was no significant difference between the deaths in the two sexes.

It is now realised that such estimates of prenatal mortality in polytocous species are possible because, usually, embryos that die are reabsorbed *in situ* without disturbing the course of gestation of the remaining embryos. In monotocous mammals abortion is the usual sequel to embryonic death, but in polytocous species it is rare unless the foetuses die near full term. Descriptions of the "spontaneous" disintegration of embryos *in utero* are given by Strahl and Henneberg (1901 and 1902), Henneberg (1903), Meyer (1917) and Fortuyn (1920) and, recently, Kerr (1947) has given an account of such disintegration induced in mouse embryos by treatment of the mothers with subcutaneous colchicine.

Both percentage prenatal mortalities and abnormalities of development vary from species to species and from strain to strain of single species. Much of the variation can be attributed to genetic differences (Gruneberg, 1943 and 1947). That inheritable lethal or sublethal factors can cause death or abnormalities of zygotes is, of course, well known. The classical instance is that of the lethal factor (or factors) in the yellow mouse (Cuénot, 1908). Cuénot's original work and that of Ibsen and Steigleder (1917) provided the evidence for the death *in utero* of homozygous yellow mice. Kirkham (1919), in more extensive investigations which included all stages of pregnancy in such mice, found abnormal morulae and blastocysts which were undergoing phagocytosis at the time of implantation. He also found similar degenerating blastocysts in "sickly" females of presumably normal stocks—an example of phenotypic simulation of the lethal genotypes! Many other lethal genes are now known in mammalian material, e.g. in other mouse strains

(Little, 1920 ; Dunn, 1937), in cats (Jones, 1922), and in horses, dogs, sheep and cattle (Mohr, 1929 ; Wriedt, 1930). As these are examples of recessive inheritance, fertility is reduced by inbreeding. Thus in extensive experiments with inbreeding in guinea-pigs Eaton (1932) was able to demonstrate a decrease in fertility associated with an increase in prenatal mortality. In certain rabbit strains Hammond (1928) found that foetal atrophy was inherited as a maternal character. The does carrying this character produced the same number of atrophic embryos irrespective of the strain of the buck. In such instances death of the embryos may be due to a deficiency of some substance in the maternal blood. In the human subject a complete genetic analysis is not available, but Streeter (1930) has produced evidence which seems to indicate that foetal abnormalities and especially atrophies have a genetic basis.

As Hammond has shown, when hyperovulation in the normal rabbit is induced experimentally the number of young finally produced is approximately the same as that following normal ovulations. This appears to indicate that some substance is present in the maternal blood the amount of which determines the number of young that are carried to term.

General environment and nutritional conditions during gestation are also concerned in prenatal death and there is now an imposing literature on the effects of vitamin deprivation in the causation of foetal mortality and abnormality (Evans and Bishop, 1923 ; Urner, 1931 ; Hale, 1935 and 1937 ; Mason, 1939 ; Warkany and Nelson, 1942 ; Warkany *et al.*, 1943 ; Warkany, 1944 ; Warkany and Schraffenberger, 1946 ; Noback and Kupperman, 1944).

The great extent of prenatal mortality in exceptional instances is well demonstrated by the studies of Brambell (1942, 1944) and Brambell and Mills (1944 and 1947a and b) and Allen *et al.* (1947) on reproduction in wild rabbits in Caernarvonshire. These workers have shown that approximately 60 per cent. of all litters in this series are completely lost, mostly about the twelfth day of gestation. As there is a loss of 10 per cent. of ova in the surviving litters only 36 per cent. of ovulated ova survive at the time of birth. When necessary allowances are made these mortality figures are fairly constant from year to year. They do not seem to hold for rabbits in other parts of Great Britain. Brambell's (1944) paper should be consulted for a discussion on the possible cause, physiological, pathological or genetical, of this extraordinary mortality. Owing to the constancy of the phenomenon in the population studied, he inclines towards a genetical explanation.

Incidentally, Brambell has directed attention to the fact that if all the embryos in polytocous species die almost simultaneously and are reabsorbed *in situ*, the effect on any estimate of prenatal mortality is equivalent to abortion in a monotocous species where such estimates are very difficult in a random population. He points out that estimates of prenatal mortality based on counts of corpora lutea and embryos in samples including all visible stages of pregnancy are liable to be extremely misleading. In the Caernarvonshire rabbit data, for example, such an estimate would disclose a mortality of only 24 per cent. Brambell and Mills (1944) have suggested a method of fractionisation of the data, according to the stage of development of the embryos, into groups each of which covers a period nearly commensurate with the time required for the complete reabsorption of a dead embryo at that stage. The mortality in that group is then estimated separately. If a significantly higher mortality is found in any of the earlier groups than in the later groups the only

explanation is a postulation of the loss of whole litters and their disappearance from the samples comprising the later groups, for the discrepancy between the number of corpora lutea (which persist throughout gestation) and of embryos represents the whole of the loss of ova from the time of ovulation. As Brambell (1944) points out, "any estimate of foetal mortality based on samples not grouped according to stage is open to serious criticism and may be very misleading." In more recent papers (Allen *et al.*, 1947; Brambell and Mills, 1947a and b), Brambell and his co-workers have analysed prenatal mortality in wild rabbits yet further and have shown that it is widespread throughout Britain. They have also shown that in tame rabbits fibrinogen, of maternal origin, is present in the yolk-sac contents. In the embryos of strains of rabbits with a high prenatal mortality the fibrinogen is converted into a clot of fibrin which, it is suggested, may be causally related to the mortality. Brambell *et al.* (1947 and 1949) have also demonstrated the presence of albumin, and α , β and γ globulins in the yolk-sac fluid in similar proportions to each other as in the maternal plasma. (For review see Brambell, 1948.)

It is very interesting to compare Brambell's results in the wild rabbit with those of one of his pupils, Perry (1945) on the wild brown rat. Perry gives a full account of earlier work on prenatal mortality in the rat. In his material the average number of ova ovulated was about 10, and about 60 per cent. of the litters suffered some loss before reaching full-term. The number of rat ova which did not reach term was probably about 25 per cent. It will be seen that the position in the brown rat is markedly different from that in the wild rabbit studied by Brambell. A relatively high proportion of the ova is lost, but few entire litters are lost. The rat prenatal mortality is apparently randomly distributed with regard to stage of gestation. If there is any tendency to lose whole litters at a particular stage of gestation it is owing to failure to implant properly and there is no evidence of a critical stage after the embryo has become attached. Ranson (1941) has provided data on prenatal mortality in the vole, *Microtus agrestis*. By palpation of the embryos he found an intra-uterine mortality of 12.07 per cent. As 14.2 per cent. of the members of the litter cast alive at term died during the 14 days between birth and weaning the accumulated loss for the 35 days of existence in this vole is at least 32.28 per cent.

In a series of twelve early human ova Hertig and Rock (1944) found that seven were normal and five showed pathological changes. Four of the pathological ova showed varying degrees of hypoplasia of the trophoblast. The ova were implanted in an apparently normal endometrium so it is presumed that the abnormal development is due to intrinsic defects in the ovum. The striking feature of the fifth abnormal embryo is the shallowness of its implantation. The embryonic rudiment is apparently normal, but Hertig and Rock consider it highly probable that the embryo would have been aborted at an early date. Boyd and Hamilton (1950) have reviewed the literature on abnormal human development. For a full account of chemical teratogenesis in vertebrates the reader is referred to Ancl (1950).

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CHAPTER 15

PLACENTATION

By E. C. AMOROSO

I—INTRODUCTION

The preceding chapter has already dealt with the structure and early development of the vertebrate egg and reference has also been made to the formation of the foetal membranes, implantation, and the nature of early trophoblastic activity. Quite as important for an understanding of the functional interplay between the parent and offspring which is associated with viviparity, are the data concerned with the structural differentiation of the foetal-maternal connection. These data have been recorded for several vertebrate classes as well as for certain invertebrates.

This chapter will attempt to outline our present knowledge of these structural adaptations and to show, wherever possible, what bearing they may have upon the general interpretation of placental function. For the sake of completeness some special cases of ovoviviparity and larval compression are included, but receive slight consideration because they do not, in the strictest sense, develop all the characteristics of a true placenta. Again, other phases of the subject, of primary significance, are briefly alluded to because they are discussed at length in other chapters.

In compiling the material, free use has been made of the subject matter of Chapter X in the second edition. The illustrations, all of which are either new or redrawn, have been selected for their value in demonstration and are intended to assist the first steps in orientating those who may not be familiar with the subject of placental histology. For points of historical interest that must necessarily be omitted from the present discussion, to conserve space, the reader may consult the second edition, and the book on "A History of Embryology" by Needham (1934).

As regards the bibliographical references, these have been given as far as conveniently possible and authority is cited for the most important points. It should be noted, however, that no effort has been made to abstract all the voluminous literature that has accumulated in the last twenty-five years in order to document every paper which has added data to the field. This would have doubled a task already great and would have added little that is of much value except to the specialist, who should, in any case, turn to the excellent bibliographies that are available, and not to a general working account.

Reference should be made at the outset to several excellent treatments which cover in more detail important phases of placentation, and supplement the pioneer and classical studies, such as those of Turner (1876a), Duval (1889-1895), Hubrecht (1889-1908), Selenka (1883-1903), Strahl (1906a), Robinson (1904), Assheton (1906) and Jenkinson (1913). The rich and varied data on human and

comparative placentation are summarized by Krölling (1927) and by Grosser in "Frühentwicklung, Eihautbildung und Placentation des Menschen und der Säugetiere" (1927). In this and in three previous monographs (1909, 1910, 1925a) will be found adequate statements of Grosser's views upon the relationships of the various types of placentae. The biology of the placenta is reviewed by Mayer, Vogt and Seitz (1929), and also excellently by Wislocki and his associates in several important contributions (1920-1947). In a recent publication Hill (1932) discusses the developmental history of the primates, while the development of the placenta from implantation to full term in several mammalian orders is reviewed by de Lange (1933). The most recent summary in the field of the comparative morphogenesis of the foetal membranes is that of Mossman (1937). He has brought together most of the available information on this subject and has, in addition, provided the most uniform and complete series of diagrams illustrating the subject of comparative placentation.

General Definition of the Term

The term "placenta" was introduced by Realdus Columbus (1516-1559), when he described the human placenta as an "affusion" of residual material in the form of a circular cake (placenta), and in this restricted sense it continued to be used, by others of his time as well as later, to describe the discoidal placenta of man, the rabbit, rat, mouse and guinea-pig. Only gradually has it acquired its present more general meaning, and as the term is now used, it includes all sorts of mammalian placentae as well as those placenta-like connections seen in certain invertebrates and some lower chordates. It has been defined as "any intimate apposition or fusion of the foetal organs to the maternal (or paternal tissues) for physiological exchange" (Mossman, 1937). In respect to essential accuracy this definition is satisfactory; nevertheless when we speak of "the placenta" we commonly have in mind a more restricted use of the term, i.e. as equivalent to the *chorio-allantoic placenta* such as is found in Mammals and Reptiles and in which the chorion is vascularised from the allantois and actually fuses with the uterine wall to form a fairly complex structure. However, in the uterine development of some vertebrates, e.g. a few sharks, some snakes and lizards, and most marsupials, it is a portion of the yolk-sac and the intervening chorion which forms the primary placental connection with the maternal tissues. In such cases the physiological processes are carried out by means of a vascular (*chorio-vitelline*) or non-vascular yolk-sac placenta.

Viviparity and Ovoviviparity

If we attempt to evaluate the meaning of all the features in the reproductive processes of mammals which distinguish them from the lower vertebrates we may sum them up in one word—protection. The retention of eggs during their development within a brood cavity or pouch is primarily a protective function. This protection conferred by viviparity reaches its climax in mammals, for with the exception of the duck-billed platypus (*Ornithorhynchus*) and the spiny ant-eater (*Echidna*) which still lay eggs in true reptilian fashion, all existing mammals are viviparous and bear their young alive. As a general rule, only a small number of

eggs are shed and these are retained inside the mother's body giving them additional protection, and in most cases there is a well-developed mechanism (placenta) for the nourishment of the young embryo within the uterus. Bigger, if fewer, offspring seems to have been the mammalian trend—quality not quantity.

Internal gestation was not, however, a new discovery by the mammalia. Examples of it occur among the invertebrates, and all three classes of the cold-blooded vertebrates contain live bearing forms. But whereas the majority of these are considered to be ovoviviparous, there are some which are viviparous and which, in addition, develop vascular uterine structures suggestive of the mammalian placenta. The distinction is based on the difference between live bearing forms which retain an egg with enough yolk to carry the embryo through its development and those in which there is an intimate nutritive connection between mother and offspring. The latter are considered truly viviparous. Thus some viviparous sharks for example, develop a yolk-sac placenta, while certain lizards and a few snakes have been found with both a yolk-sac and an allantoic placenta. Among insects too, there are attempts at a placenta (Hansen, 1894; Heymons, 1912; Hagan, 1931), while in the same category belongs also the placenta-like organ of the primitive worm-like arthropod *Peripatus edwardsii*. This is also the case in some Tunicates (e.g. *Salpa democratica*) and, as already indicated it reaches its climax in the intimate and extensive organic relationship between embryo and uterine wall among Eutherian Mammalia. The only vertebrate class which contains no viviparous members is Aves. For further details, see Wunder (1931, 1932, 1934, 1937).

II.—ADAPTATIONS FOR VIVIPARITY IN FISHES

Selachian Ovoviviparity

Among the elasmobranchs, numerous examples of the birth of living young exist in which the embryo within the uterus is still nourished by the yolk in the yolk-sac. There are, however, considerable variations in the composition of the uterine fluids bathing the egg cases while the embryo develops, indicating that, in these ovoviviparous forms the uterine wall already plays an important part in providing food material for the young individual by its glandular activity (Ranzi, 1934). In some, the secretion of "uterine milk" (see p. 152) is accomplished with little or no gross alteration of the uterine wall while in others a more or less complete reorganisation of the uterine lining occurs. This latter may be smooth and may contain many mucus secreting cells (e.g. *Mustelus vulgaris*); or, project into longitudinal folds which are invested with a stratified epithelium containing few mucus secreting cells (e.g. *Acanthias vulgaris*), or grow out into villi (*Torpedo ocellata*, *Pteroplatea micrura*, *Myliobatis nieuhofii*, *Trygon walga*). *Pteroplatea* develops long villi in the walls of the uterus which enter the spiracle of the embryo and extend down into the oesophagus. These extensions of the mucous membrane of the uterus, to which the term "trophonemata" is applied (Wood-Mason and Alcock, 1891), are beset with tubular glands so that their secretion reaches the alimentary canal of the young fish directly. They also probably assist in respiration. In *Torpedo* the villi are more numerous but not so extended. The epithelium is here one-layered and there are many glands but no mucus secreting cells; while in *Trygon* there are as many glands as before, but the epithelium is stratified, and the

villous condition reaches its climax. These thick-set papillae secrete the uterine milk and there is no connection between foetus and mother. In *Myliobatis nieuhofti*, Alcock (1890) states that the uterine papillae are less attenuated and the whole uterine mucosa forms a superficial milk gland.

Before leaving the ovoviviparous selachians, something must be said about the curious mode of nourishment of the young of *Lamna cornubica* studied by Shann (1923). Shann has shown that following the early absorption of the yolk, the nutrient material is derived from the ovary in the form of immature eggs or partly degenerate ovarian tissue which are taken up by the oviduct and passed into the uterus where they are swallowed by the embryos. Feeding like this continues for over a year, when the cardiac stomach assumes gigantic proportions and is then called the "yolk-stomach." This pronounced modification of the stomach closely parallels the temporary elongation and expansion of the hind-gut seen in the embryos of Anablepid fishes, and it is possible that it provides an important pathway for the absorption of nutritive materials from the parent, although conclusive proof of this is lacking (see p. 133).

The Selachian Yolk-sac Placenta

Turning now to the true viviparous forms, we find that in some species there is a specially modified portion of the yolk-sac which comes in contact with the uterine wall and through which food material and oxygen are absorbed from the mother and waste probably removed. This is, in fact, a type of yolk-sac placenta. According to Cole (1944), "it was the Danish anatomist Steno (1673) who was the first, after Aristotle, to claim that the connection was a functional placenta, by the activity of whose blood vessels soluble food materials were transferred from the parent to the cavity of the yolk-sac, and thence by its stalk to the gut of the embryo." It has been described in *Mustelus laevis* (J. Muller, 1840; Ranzi, 1932, 1934; Cate-Hoedemaker, 1933), *Carcharias glaucus* (J. Muller, 1840; Calzoni, 1936), *Carcharias melanopterus* (Alcock, 1890), and *Scoliodon sarrakozahi* and *S. palasorrah* (Mahadevan, 1940).

In *Mustelus laevis* the embryos are separated by compartments and in the placental area, the uterine wall develops villi which interlock with grooves on the surface of the yolk-sac. In the paraplacental region the yolk-sac consists of endoderm, vascular extra-embryonic mesoderm and ectoderm. In sections the endoderm cells are cuboidal, those of the mesoderm are spindle shaped and the outer cells are tall columnar (Fig. 15. 1 a). The uterine epithelium is here many layered and some of the cells are said to produce a mucoid secretion. If we may judge of what happens now by what is known of the changes which occur in the paraplacental region as pregnancy advances, the yolk-sac placenta of *Mustelus* is formed in an exceedingly simple fashion. The epithelium lining the uterus becomes modified; the number of cell layers is reduced and the cells become cuboidal (Fig. 15. 1 b), and then fairly flat (Fig. 15. 1 c). The maternal capillaries soon penetrate and run among the epithelial cells coming to lie in many places in contact with the egg membrane (Fig. 15. 1 d). The ectoderm of the yolk-sac is also modified; the cell outlines disappear and the epithelium is reduced to a narrow syncytial frond which soon disappears altogether (Fig. 15. 1 d). The foetal mesoderm is also greatly reduced, thus exposing the capillary blood vessels to the

egg membrane. In this way the two circulations are brought surprisingly close to one another at these points and subserve the more direct interchange of substances—especially the gaseous exchange—between the mother and her offspring.

An important function of the yolk-sac placenta is undoubtedly that of absorption. Considerable absorption of organic material probably takes place; fat in particular being made available by this direct route. The significance of the Elasmobranch histotrophe has been well described by Ranzi (1935) and is summarized by Needham (1942).

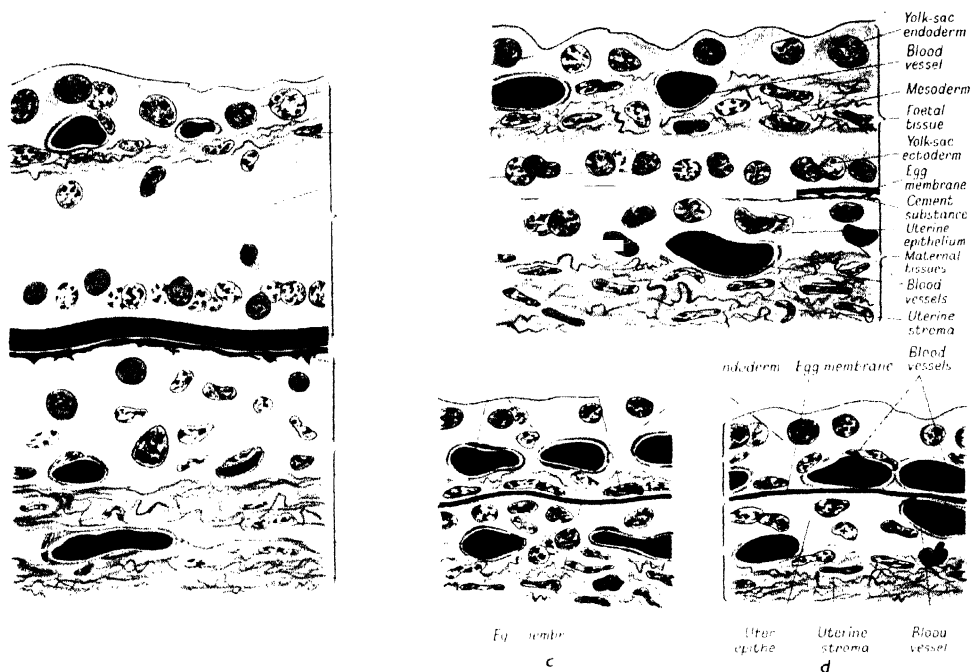


Fig. 15. 1—Stages in the development of the yolk-sac placenta in *Mustelus laevis*.

(a) Section through the parapental region of the yolk-sac and uterine wall. 600.

(b, c, d) Sections through the yolk-sac placenta at three successive stages of its development. 600.

For explanation see text. (Redrawn and modified from Cate-Hoedemaker.)

As in other viviparous Elasmobranchs the embryos in *Scoliodon* are lodged in separate compartments of the uterus, the mucosa of which is raised in places and then invaginated in the form of a single long trophonematous cup with a rich vascular supply. Both the yolk-sac and the part of the trophonematous cup to which it becomes connected undergo important changes resulting in an almost complete union between the two structures. In most species of *Scoliodon* special structures, the appendicula, are developed on the placental cord. These are simple, long and thread-like, and rarely branched in *S. sarrakowah*, much branched in *S. palasorrah* and very short and branched in *S. walbechmi*. They help in the absorption of the uterine secretions. An important feature of the yolk-sac of *S. sarrakowah* is that the cavity of the yolk-sac is not filled with yolk granules but

with a network of cellular strands saturated with circulating blood. The yolk-stalk is likewise obliterated, forming a placental cord, so that in the ripe placenta the connection between mother and offspring becomes purely vascular as in mammals.

Teleostean Oviviparity and Viviparity

We come now to a special case of much interest, that of the embryos of viviparous Teleosts, to which Turner (1933, 1940a, 1940b, 1940c, 1940d) has devoted much study. From the multitude of varied phenomena that he has recorded, two salient facts stand out. First, viviparity is, with few exceptions, concentrated in a few orders, being especially well developed in the order Cyprinodontes in which all genera and species of the four families *Poeciliidae*, *Anablepidae*, *Goodeidae* and *Jenynsiidae* have viviparity more or less well developed. Secondly, that Teleostean fishes constitute yet another case of apparent succession of adaptations

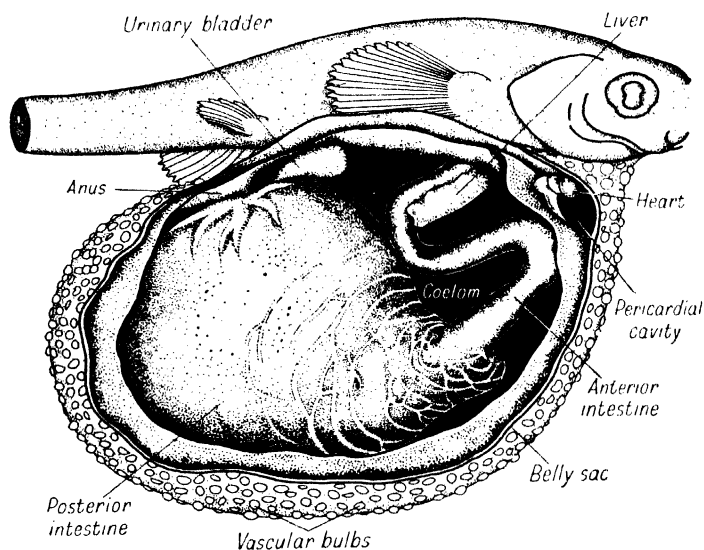


fig. 15. 2—Partially dissected embryo of *Anableps dowei*, showing relations of pericardial cavity, coelomic cavity and expanded posterior intestine. (Redrawn from Turner.)

associated with the birth of living young which range from little or no alteration of the oviparous types of structures (e.g., the guppy, *Lebistes reticulatus*), to conditions in which the yolk-sac has degenerated and a series of temporary but unique structures occur by means of which the embryos respire and obtain nutritive materials.

Poeciliidae and Anablepidae. In *Heterandria formosa* (Turner, 1940a; Fraser and Renton, 1940; Scrimshaw, 1944, 1945), as in all poeciliid and anablepid fishes, the embryos are retained until parturition within the follicles of the single median ovary. Following fertilisation the epithelium of the follicle is transformed into a syncytium, the fertilisation membrane disappears and the pericardial sac of the embryo becomes so enormously extended as to enclose completely the [head].

An extensive portal capillary network develops on this expanded portion of the somatopleur so that its whole surface apposing the follicular syncytium is highly vascularised. Where the two are in contact, the follicular epithelium is thrown into low folds or villi and since the latter have likewise developed an extensive vascular system, an association between the two circulations comparable to the mammalian epitheliochorial placenta is formed. For the complex composed of the follicular wall with its villi, the follicular space and the adjacent vessels of the portal system covering the belly sac, Turner (1940a) has proposed the term "*follicular pseudo-placenta*."

In Anablepid fishes, this type of follicular pseudo-placenta is best developed in the two closely related species *Anableps anableps* and *A. dowei*. In addition, Turner (1940b) has shown that the adoption of viviparity by these typical anablepids has been accompanied by new adaptations. The most important of these relate to a pronounced modification of the gut in the form of a temporary elongation and expansion of the hind gut and the development of vascular bulbs on the portal vein tributaries (Fig. 15.2). He believes that these may provide an additional pathway for the absorption of nutritive materials from the parent organism, the fluid drawn into the gut being derived from the follicular liquor and not from the ovarian fluids as in the *Goodeidae* and *Jenynsiidae*.

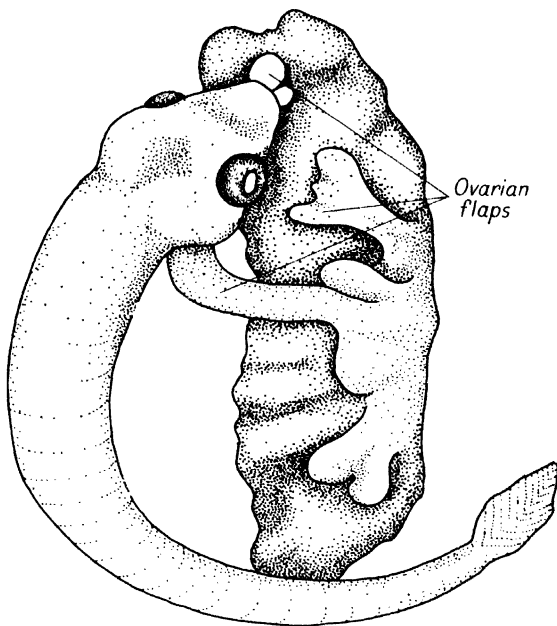


Fig. 15.3 Late stage in the development of *Jenynsia lineata*, showing the attachment of the embryo to ovarian flap. The ovarian tissue extends through the opercular opening into the pharyngeal cavity and a small portion protrudes through the mouth. (Redrawn from Turner.)

Goodeidae and *Jenynsiidae*. Whereas in the *Poeciliidae* and the *Anablepidae*, the embryos are retained during gestation within modified ovarian follicles, in the Goodeid and Jenynsiid fishes the embryos are discharged from the ovarian follicles into the ovarian cavity where they undergo their development.

Correlated with the development of the embryos, the ovarian epithelium, and with it the stroma, become modified and undergo progressive changes. A stage in which the ovarian epithelium becomes highly secretory in character is followed by one in which masses of these cells are desquamated. It seems that these masses together with material derived from the autolysis of non-viable embryos—a common phenomenon in Jenynsiid fishes—enrich the ovarian liquor and are directly utilised by the surviving young. In the stroma, the blood vessels become relatively quite numerous and many of the minor branches, especially in the

Jenynsiidae, are said to enter the overlying epithelium where they pursue an intraepithelial course. As the embryos mature the ovarian tissue regresses considerably and in late gestation is replaced by a series of highly vascular folds which partially fill the ovarian cavity and come in contact with the gills, sometimes even entering one of the opercular clefts. Ovarian flaps are, however, comparatively inconspicuous in the *Goodeidae*, but in the *Jenynsiidae* they appear to be almost universally present and with their rich vascular folds are in a position to promote respiratory and nutritive exchanges between the maternal blood and the gills of the embryo with which they are in contact (Fig. 15. 3). Indeed, this arrangement in the *Jenynsiid* fishes may be looked upon as a potential *branchial placenta*, for here the epithelium of the gills and the ovarian epithelium are closely approximated and when it is realised that over a considerable area only the single layer of branchial epithelium plus a thin cytoplasmic plate of the ovarian epithelium separate the two blood streams, the intraepithelial capillary plexuses acquire greater significance as a means of exchange between mother and offspring.

In the *Goodeidae* a different device is utilised for furnishing the embryos with materials for further growth while they are retained within the ovarian cavity. Extensive rosette shaped proctodeal processes, the "trophotaeniae," are developed. These are believed to supplement the respiratory functions of the gills by replacing, in some cases (e.g. *Zoogonecticus quitzooensis*), the pericardial sac functionally. Nutritive substances are also apparently absorbed by the gut from the fluids drawn into the pharynx and swallowed—a device employed to some extent by the embryos of all viviparous Cyprinodonts.

III.—LARVAL ADAPTATIONS IN AMPHIBIA

Under this heading we include what are really only special cases of ovoviviparity and larval compression, for no true viviparity occurs. Practically all Amphibia are oviparous, and in addition most of them lay their eggs in water, because of the fundamental need of an aquatic environment for the metamorphic changes associated with the development of the young. However, a small minority, including representatives of all three orders, Urodela (e.g. *Salamandra atra*), Anura (e.g. *Nectophrynoides tornieri*) and Gymnophiona (e.g. *Typhlonectes compressicauda*) retain the eggs in the oviduct, where they undergo development.

Of these, however, the urodele *Salamandra atra*, is the only ovoviviparous form in which continued nourishment of the embryo, after it has used up its own yolk supply, accompanies maternal incubation. Here the young are reduced in number by cannibalism within the uterus, and only the few which survive this uterine struggle are born. There is a definite utilisation not only of the yolk debris of the non-viable eggs, but also of large quantities of red blood corpuscles derived from the extensive haemorrhages of the uterine wall. The former is also considerable, since a relatively large number of ova (40–60) are fertilised and begin their development but the number of embryos that survive to the close of the period of gestation is relatively small (1–4). The larvae in this species possess long plume-like external gills during their sojourn in the oviduct.

From the standpoint of the present chapter, however, the most interesting cases are those of the two anurans *Pipa dorsigera* and *Nototrema marsupiatum*. In the former the eggs are spread at fairly equal intervals over the female's back and

each ovum is pressed into the skin and finally comes to lie in a deep cavity which is closed by a lid, and in which the young develop. This includes a modified tadpole stage which is passed entirely within the pouch. The young toads are born eventually after about 82 days (Bartlett, 1896). Fig. 15. 4 shows the later stages of development of *Pipa dorsigera*. It will be observed that each cavity consists of an invaginated epidermal pouch with highly vascular partitions between the eggs. Moreover, the larva is characterised by a special modification of the tail which is expanded and very richly vascularised and which doubtless serves as a placenta, mediating respiratory and possibly nutritive exchange with the maternal tissues. The origin of the operculum is uncertain, but the probability appears to be that it is produced by the remnant of the egg envelope itself, reinforced by epidermal secretions.

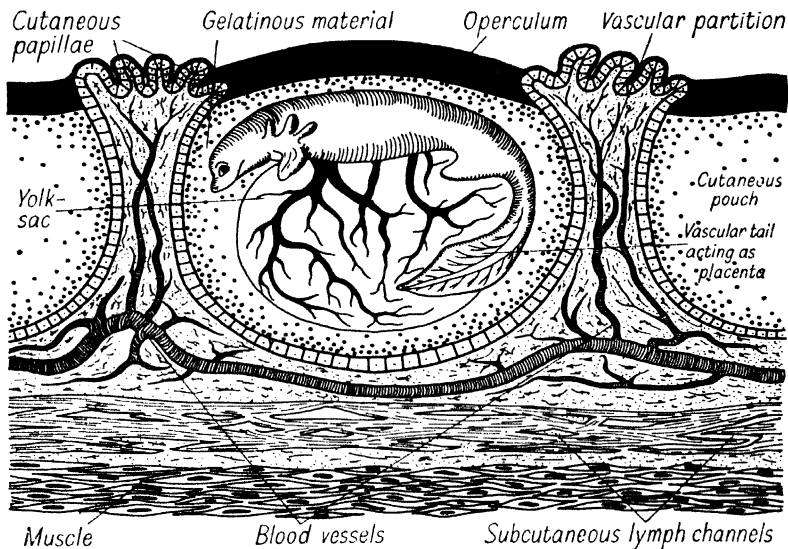


Fig. 15. 4.—Late stage in the development of the Surinam toad (*Pipa dorsigera*), showing embryo with expanded leaf-like tail lying in cutaneous pouch.

In the genus *Nototrema*, the eggs are stored in a specially formed dorsal pouch of the integument which opens to the exterior just in front of the cloacal aperture. Just how this is brought about remains unknown. There is, however, the suggestion of Boulenger (1895) that it occurs in response to the presence of eggs on the animal's back. This probably is true, but it furnishes little evidence as to the controlling mechanism. In this pouch the eggs are carried, in some species (*N. marsupiatum*) until the tadpoles emerge or as in *N. oviferum* until after the period of metamorphosis (Weinland, 1854), and in most species enormously expanded and highly vascular, leaf-like or bell-like gills are developed in connection with vascular ridges of the skin of the interior of the marsupial pouch of the adult.

In the various modifications of amphibian development dealt with in the preceding paragraphs, it is clear that while the all important source of food supply is the relatively dry yolk provided by the maternal organism (in the egg), this

attempt to obtain extra nourishment from the parental tissue, which has led to no extraordinary success, is, in a way analogous to placentation and tends to avoid, to some extent, the necessity for the double mode of life—(and a double set of adaptations) to which the name of the group refers.

IV.—ADAPTATIONS FOR VIVIPARITY IN REPTILES

In contrast with the Amphibia, reptiles have evolved a type of egg which can be laid on land. The egg is much larger than in Amphibia and the young are, in most cases, provided with a supply of food which is sufficient to carry them through to a stage where they can make their own way on land. An early development of the yolk-sac supplies the store house for the reserve material. A still more character-

istic feature is the development of two new membranes by the embryo itself, the amnion and the allantois and in the egg laying forms the whole egg is surrounded by either a soft (as in snakes) or a hard chalky shell (as in the alligator). The shell, however, is porous and over a considerable part of its inner surface is in contact with a rich system of small allantoic vessels, so that a ready interchange of respiratory gases between the blood and the external air is provided for. But, whereas egg laying is the more common, in some snakes and lizards the eggs are retained in the oviduct until the young are ready to be born.

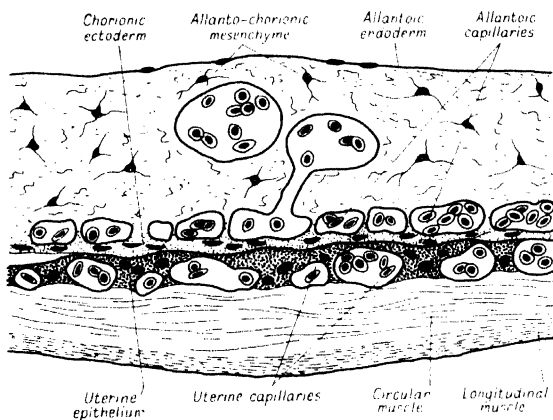


Fig. 15. 5—Section of mature chorio-allantoic placenta of *Egernia cunninghami*, showing the maternal capillaries more or less exposed at the maternal placental face. (Redrawn from Weekes.)

It has also been suggested that the reproduction of Ichthyosaurs must have been of a similar nature, with the possibility that some maternal connection was formed.

It has long been known that the lizard *Chalcides tridactylus* develops both a yolk-sac and an allantoic placenta (Giacomini, 1891) and these same characters have been detected in several Australian lizards and in certain Australian snakes.

The reptilian yolk-sac placenta.

The detailed descriptions of Giacomini (1891) and Cate-Hoedemaker (1933) for the Italian lizard *Chalcides tridactylus*, and of Weekes (1930, 1935) for several Australian lizards, and Boyd (1942) for *Hoplodactylus maculatus* indicate that in these reptiles the yolk-sac placenta is like that of the placental dogfish and involves enlarged chorionic ectodermal cells around the lower part of the egg, lying opposite to enlarged secretory uterine epithelial cells. The thickened chorionic epithelium does not extend uniformly over the yolk-sac but in irregular patches, so that there may be thin areas interspersed among the thick areas. In some of the larger lizards—the *Egernias* and the *Tiliquas* for example—both the uterine wall and the

omphalo-chorionic membrane may be thrown into interlocking folds, or the uterine wall alone may be folded (Weekes, 1930), or, as in *Hoplodactylus maculatus* both layers may be pleated and folded but do not interlock (Boyd, 1942). However, even where the two tissues are in closest contact there is no invasion of maternal tissue by the foetal tissue and placentation remains quite simple.

The reptilian chorio-allantoic placenta.

Among the Reptilia, Weekes (1935) distinguishes three main types of chorio-allantoic placentation.

(I) The extremely simple type (Fig. 15. 5) which Flynn

(1923a), and Weekes found to be widespread among Australian lizards and in which the extreme reduction of the uterine and chorionic epithelial tissues leads to a very close approximation of the maternal and foetal blood streams. This type

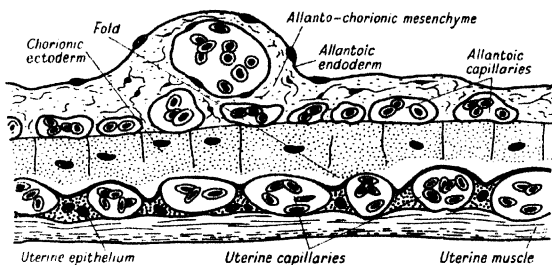


Fig. 15. 6—Section of mature chorio-allantoic placenta of *Lygosoma* (*L.*) *ocellatum*, showing apposition of maternal and foetal tissues. For explanation see text. (Redrawn from Weekes.)

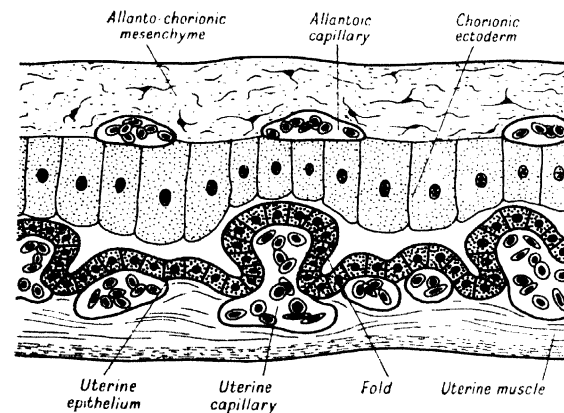


Fig. 15. 7—Section of young chorio-allantoic placenta of *Egernia entrecaesteuxi*, showing folded maternal placental face. The larger size of the folds is noticeable when they are compared with those of *Lygosoma* (*L.*) *ocellatum* shown as Fig. 15. 6.

of the genus *Lygosoma*. The most striking feature of the placenta in these forms is the extreme thinness of the uterine wall. In the ripe placenta the intact maternal capillaries instead of coursing level with the maternal placental face, bulge at the surface of small uterine folds, the grooves between which are lined with glandular epithelium. The chorionic epithelial cells are enlarged and over most of the placental region interlock with the shallow maternal grooves, often extending cytoplasmic processes which penetrate for some distance between the uterine epithelial cells.

has also been described for *Chalcides ocellatus* (Giacomini, 1906) and *Hoplodactylus maculatus* (Boyd, 1942) as well as in the snakes *Demisonia superba* and *D. suta* (Weekes, 1929). When the placenta is mature the maternal capillaries are pressed against and often indent the bases of the overlying epithelial cells. It is also noteworthy that the chorionic ectoderm cells are furrowed by the bulging allantoic capillaries, so that often only the thinnest cytoplasmic membranes separate the two circulations.

(II) The second type (Fig. 15. 6) is also simple and has been described in several species

(III) The third type is the most highly specialised and has been described for *Chalcides tridactylus* (Giacomini, 1891), *Lygosoma (Liolepisma) entrecasteauxi* and *L. (L.) weeksae*. Here the placental region is roughly elliptical in shape and the folded maternal tissue immediately overlies the main longitudinal uterine blood vessels. The uterine folds are enlarged and complex and indent the foetal placental face (Fig. 15. 7). Each is filled with capillaries of various sizes and is lined by a single layer of enlarged, cuboidal, ciliated epithelial cells. The ectodermal cells of the chorion are likewise ciliated and enormously enlarged and immediately overlie the allantoic capillaries. It must be emphasised, however, that the contact between maternal and foetal tissues is, in all cases, more or less epitheliochorial and compares therefore with *Mustelus laevis* in the Selachian series.

V.—THE MAMMALIAN PLACENTA

The mammalian ovum, in all except the Monotremata, is small and does not contain a sufficient supply of nutriment for the developing embryo. It is retained for a longer or shorter period in the uterus, where, by special modifications of the uterine mucosa and a part of the ovum, a placenta is formed, and a transmission of nutriment from the mother to the embryo is made possible. Accordingly, the normal mammalian placenta may be defined "as an apposition, or fusion, of the foetal membranes to the uterine mucosa for physiological exchange" (Mossman, 1937). The changes in the maternal and embryonic tissues vary greatly in the several orders, and even in groups of the same order, but in all they are sufficiently complicated to render their explanation a matter of great difficulty. It is doubtful if any anatomical structure has given rise to keener or more prolonged controversies than the placenta.

In this section we must be content with a review of the processes occurring in several Mammals which have been more particularly investigated, without straining to find how such processes have arisen in the course of placental evolution. The arrangement of the placenta types is in accordance with Grosser's classification, and an attempt has been made to emphasise the progressive breaking down of the barrier between the maternal and foetal circulations. Because of limitations of space the account is based primarily, but not exclusively, upon the phenomena apparent in the domestic and laboratory mammals as well as in man and the other Primates.

Fundamental Types of Placentation

Chorio-allantoic placentation

The most typical structure coming within the scope of the above definition is the chorio-allantoic placenta, but as it is not the only structure which does so, it has been more closely defined as "a portion of the chorion vascularized by allantoic vessels, it is intimately apposed to or fused with, the uterine mucosa and it serves as a medium for physiological exchange" (Franklin, 1944). All Eutherian mammals and some Marsupials, notably *Perameles* (bandicoot) develop such a placental attachment.

Yolk-sac placentation.

The chorio-allantoic placenta is not the only means of contact between the foetus and mother. When the blastodermic vesicle becomes adherent to, or sinks

into, the uterine mucosa, the wall of the yolk-sac in some orders becomes intimately related to the uterine mucosa and is nourished by it. Even in the non-mammalian Vertebrata the latter condition has been observed; it is, in fact, a yolk-sac placenta. In mammals such a placental connection is often present as a transient supplementary structure, but it may persist, and in some species (e.g. rat) "function as an organ of exchange whose importance is not secondary to that of the allantoic placenta" (Everett, 1935). (See also Brambell *et al.*, 1947.)

The yolk-sac placenta includes the non-vascular bilaminar omphalopleure and the vascularised trilaminar omphalopleur. The former is the foetal component of the yolk-sac placenta during its early development and consists of the combination of the endoderm of the yolk-sac and the ectoderm of the trophoblast which is in apposition with the endometrium. It is the earliest structure possessing the characteristics of a true yolk-sac placenta. When invaded successively by the mesoderm and area vasculosa this bilaminar structure is converted into the vascular trilaminar omphalopleur, the foetal component of the *chorio-vitelline* placenta. Mossman (1937) defines it "as a fusion or intimate apposition with the mucosa of the primitive trilaminar yolk-sac, vascularized by the vitelline vessels (area vasculosa)." Called the "ecto-placenta" by Duval, it is believed to be more primitive than the "inverted yolk-sac placenta." It occurs in most Marsupials and is generally considered to be of greater physiological importance in those like the opossum (*Didelphys*), where it constitutes the only vascular connection between foetus and mother, than in those like the bandicoot (*Perameles*) where it functions concurrently with an allantoic placenta.

Among the Eutheria, a chorio-vitelline placenta is present as a temporary structure which ceases to exist whenever the exo-coelom extends into the area vasculosa and splits it into the vascular splanchnopleur and the non-vascular somatopleur or true chorion. This separation which starts at the embryonic hemisphere spreads outwards with varying rapidity and completeness, depending on the species. In some of these, such as the pig, sheep, cow, cat and dog, the extension of the extra-embryonic coelom throughout the entire extent of the primitive blastocyst wall or omphalopleur leads to the establishment of a complete chorion and a yolk-sac vesicle with an independent wall of its own. In others, such as the horse, rabbit and squirrel the coelom does not spread so far. In the horse the chorio-vitelline

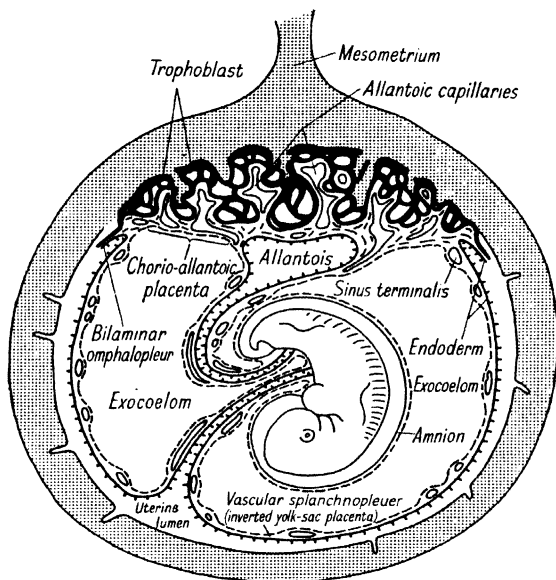


Fig. 15. 8—Diagram to show complete inversion of the yolk-sac in the rabbit and the anastomosis of vitelline and allantoic vessels.

placenta persists until a relatively late stage of pregnancy; in the rabbit the bilaminar omphalopleur, though very large at first, disappears by the limb-bud stage, while in the squirrels the bilaminar omphalopleur is large and persists till term.

An "inverted yolk-sac placenta" (Fig. 15. 8) of very different structure is found in the more primitive rodents such as the *Sciuridae* (squirrels), as well as in some Insectivora such as *Sorex* (shrew), *Tupaia* (tree-shrew), *Talpa* (mole), Chiroptera (bats) and in the armadillo among the Edentata. It "is that formed by the vascular embryonic segment of the yolk-sac (vascular splanchnopleur) inverted against the non-vascular abembryonic segment, so that in the incomplete condition (e.g. shrew, mole, bat, etc.), the bilaminar omphalopleur still separates the

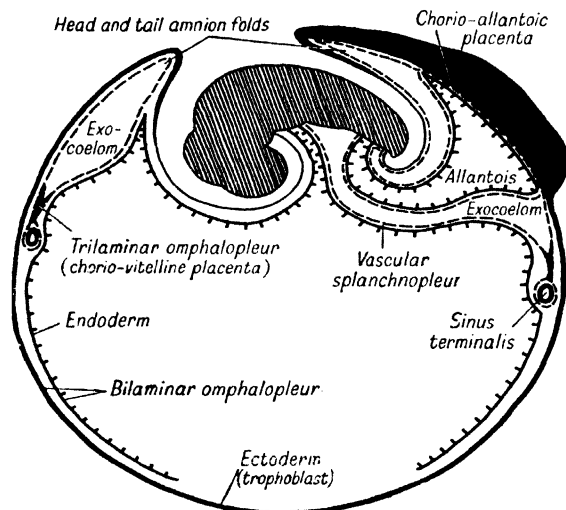


Fig. 15. 9.—Diagram to illustrate the three parts of the yolk-sac in the rabbit.

abembryonic omphalopleur or come directly in contact with the endometrium through disappearance of the intervening portion of the chorionic sac. For in none of these cases can the invaginated yolk-sac roof (vascular splanchnopleur) be considered an integral part of a placenta.

It still remains to consider the path by which the nutriment is conveyed to the embryo. In partial extension of the area vasculosa, the wall of the yolk-sac consists of three parts, each with different relations (Fig. 15. 9): (1) The non-vascular part, with a two-layered wall of ectoderm and endoderm; (2) the vascular part, where the mesoderm is unsplit, e.g. in the opossum—the mesoderm splits in its entire extent in the rabbit; (3) the part opposite the coelom. In all three parts the trophoblast is bathed by the uterine secretions after the disappearance of the zona pellucida. In the non-vascular part it is probably transmitted through the endoderm cells to the yolk-sac, whence, in turn, it reaches the embryo either by the vitelline vessels or the developing alimentary canal. In the vascular part the same may occur, or the nutriment may be conveyed to the embryo directly by the vessels of the area vasculosa. It is in this region that the foetal circulation is brought close to the maternal, and gaseous exchanges may be effected. In the

endoderm of the vascular segment from the uterine tissues, while in the complete type (e.g. rabbit, guinea-pig, rat and mouse), the bilaminar omphalopleur disappears (Fig. 15. 8) and the endoderm is in direct apposition to the uterine mucosa" (Mossman, 1937). However, if we adhere to the usual definitions, given in earlier paragraphs (and make fusion or intimate apposition a characteristic of any placenta), there is, as Wimsatt (1945) suggests, a justifiable question as to the propriety of speaking of an inverted yolk-sac placenta in those cases where the invaginated roof of the yolk-sac does not either fuse with the persistent

rat, Everett (1935) got results which he interpreted as showing that trypan blue and toluidin blue behave differently towards the inverted yolk-sac placenta and that both enter it much faster than they do the placental membrane of the allantoic placenta. Toluidin blue, he found, was instantly reduced on entering the yolk-sac placenta.

Opposite the coelom the trophoblast is lined by a thin layer of non-vascular somatopleure, through which transference of material to the coelomic cavity is possible (*see below*). This part is subsequently connected with the embryo by the allantoic vessels. When the yolk-sac is entirely separated from the outer wall, nutritive substances may also be transmitted to the coelomic cavity and then to the embryo or yolk-sac. (*See also* Brambell *et al.*, 1947.)

Chorionic placentation.

According to Mossman's views, "the so-called chorionic placenta of man and other primates is actually a chorio-allantoic placenta, since it is vascularized by the allantoic vessels and since the body stalk of Primates is definitely a homologue of the allantois." The true chorion is never vascular. However, in the Rodentia and in some other orders (e.g. Carnivora) chorionic placentation does exist independently of the allantois for a time. In the *Spermophile*, *Citellus tridecemlineatus* (Mossman and Weisfeldt, 1939) and other sciurids for example, a portion of the non-vascular, extra-embryonic somatopleure or true chorion immediately dorsal to the amnion becomes apposed or fused to the mesometrial wall of the uterus to form a transient, supplementary, non-vascular, chorionic placenta. This chorionic placenta develops independently of the chorio-vitelline placenta and obviously represents a step in the formation of the chorio-allantoic placenta since a part of it may later become vascularised by allantoic vessels. But over a considerable portion of the true chorion in the region lateral to the future chorio-allantoic placenta and between the latter and the edge of the inverted yolk-sac, contact is maintained between the non-vascular extra-embryonic somatopleur and the uterine mucosa. This area represents, therefore, a persistent region of chorionic placentation and may according to Mossman and Weisfeldt (1939) function in the interchange of materials between the exo-coelomic cavity and the uterine lumen. This persistent portion of outlying true chorion, unlike the sac-like fold of the chorion which lies dorsal to the amnion, comes into existence when the vascular, trilaminar omphalopleur of the embryonic hemisphere is split by the exo-coelom into splanchnopleur and somatopleur, its splanchnopleuric derivative being added to the invaginated roof of the yolk-sac, forming with the abembryonic omphalopleur the so-called inverted yolk-sac placenta as described on p. 140.

The mode of development of the chorionic placenta in the Carnivora differs but slightly from that encountered in the *Spermophile*. It is formed largely by de-vascularisation of the chorio-vitelline placenta and reaches its most active condition before the allantois begins attaching to the antimesometrial wall of the gestation sac. By reference to Fig. 15. 10 it can be seen how the extension of the extra-embryonic coelom enlarges the region of chorionic placentation. During most of this period the embryo is nourished by the cup-like chorio-vitelline placenta which covers the mesometrial wall of the uterus. However, the fact that the non-vascular, chorionic placenta comes into existence independently of the allantois and during the period of functional decline of the chorio-vitelline placenta opens

the possibility that it may provide a pathway through which transference of material to the coelomic cavity is possible.

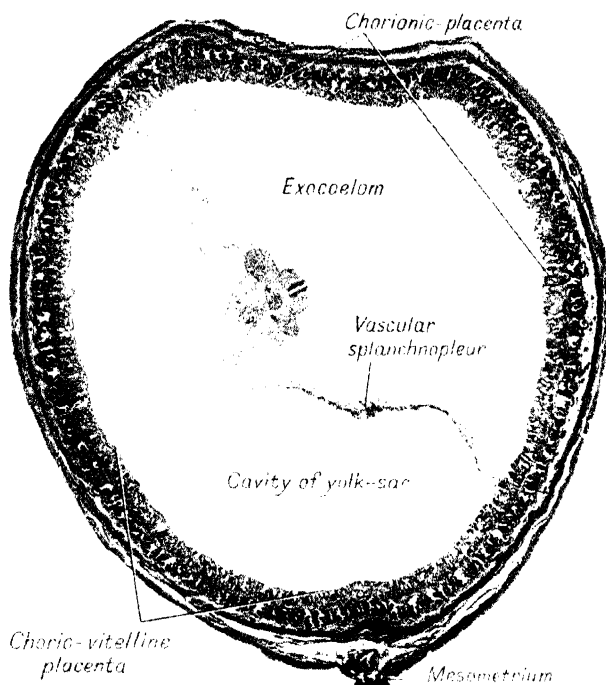


Fig. 15. 10—Section through embryonic swelling of a cat 16½ days after service, showing the chorionic placenta and the attachment of the chorio-vitelline placenta to the mesometrial wall of the uterus. $\times 5$.

Placental Classification

The main question which we have to consider concerns itself with the types of chorio-allantoic placentation. As we have seen in an earlier section, the nature and purpose of "the placenta" is to mediate the transference of metabolic materials between the mother and her offspring. In all the true mammals this always involves, on the one hand, the uterus (maternal placenta), and on the other, the chorion (foetal placenta) the outermost of the foetal membranes which envelops the foetus. This view, long ago adopted by V. Baer (1828) was reasserted by Weber (1835) and Milne Edwards (1844) and later clearly restated by Huxley (1864), Strahl (1906a), Grosser (1909) and others.

Classification by shape

Formation of "chorionic villi" characterises the union of the foetus with the uterus in all the higher mammals and since these are the organs of primary importance in affecting the nourishment of the foetus, their final distribution upon

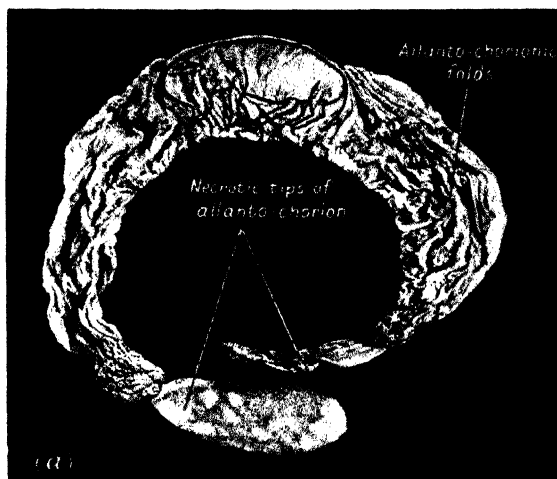


Fig. 15. 11 (a) Diffuse distribution of "villi" on the allanto-chorion of a pig removed from the uterus at the 70th day of pregnancy. Note the allantoic diverticula, $\frac{1}{4}$ natural size.

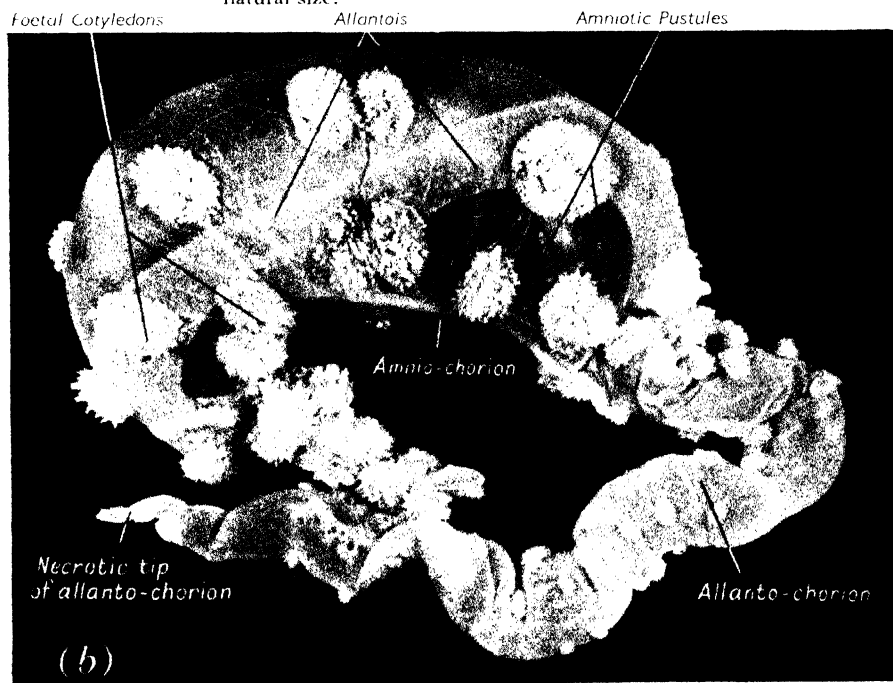


Fig. 15. 11 (b) Bovine foetus within its membranes removed from the uterus at the 90th day of pregnancy, showing linear distribution of foetal cotyledons on the surface of the chorion and the characteristic T-shaped junction of the allantois with the urachus. Note also the allantoic diverticula and the amniotic pustules. $\frac{2}{3}$ natural size.

Fig 15. 11—(a d) Types of chorionic sacs in mammals.

the surface of the chorionic sac and the varying relationships which they establish with the maternal endometrium have been utilised in defining some of the characters of the chorio-allantoic placenta. For a number of reasons, however, classification based on external configuration, etc., is not entirely satisfactory (Wislocki,

1929; Mossman, 1937); nevertheless, for convenience of description, some reference to these qualities is desirable.

The placenta shows a great diversity of form in different orders of mammals and often shows marked differences in external appearance in the same animal at different stages of pregnancy (e.g. cat, ferret, sloth and man). In shape the placenta varies from the primitive type in which the chorionic villi are distributed quite uniformly on the outer surface of the chorion—*placenta diffusa* (Fig. 15. 11 a),

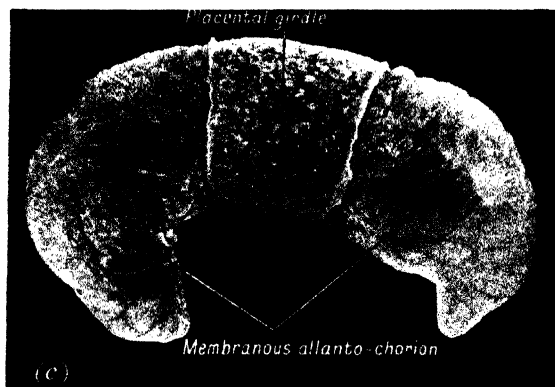


Fig. 15. 11(c) Zonary girdle of villi in the allanto-chorion of a cat at the 7th week of pregnancy. $\frac{1}{2}$ natural size.

to that in which the villous areas are restricted to form well-scattered cotyledons—*placenta cotyledonaria* or *multiplex* (Fig. 15. 11 b), a girdle-like band about the middle of the chorionic sac—*placenta zonaria* (Fig. 15. 11 c) or a disc-shaped placenta—*placenta discoidalis* (Fig. 15. 11 d). To a certain extent these conditions are characteristic of different groups; in the Perissodactyla (horse and donkey) and Bunodonta (pig), for example, the placenta is commonly diffuse; in the Pecora (Ruminants) cotyledonary or multiplex; in most Carnivora zonary, and in the Rodentia, Insectivora, Chiroptera and Primates discoidal or double discoidal.¹ On the other hand, in widely diverging groups there may be striking similarities in the shape of the placenta, while great differences may exist in closely related types. Thus, the American mole (Insectivora), the camel and musk deer (Pecora) and the Lemurs among the

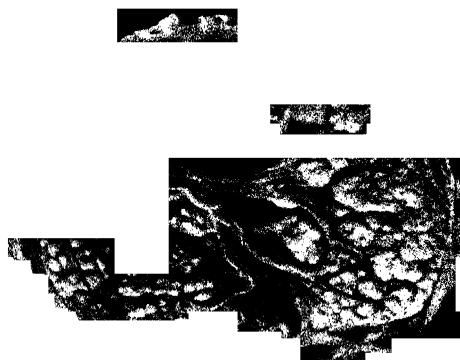


Fig. 15. 1(d) Foetal surface of the discoid placenta of a rabbit near term. $\times 2$.

¹ The bi-discoidal condition occurs commonly in platyrrhine and catarrhine monkeys and the same is true also of *Tupaia javanica* (Tree Shrew). It is interesting to note that cases are known of a double discoid placenta in man very similar to that of the monkey.

Primates have a diffuse placenta, while among the Edentata, the pangolin (*Manis*) has a diffuse placenta, the sloth (*Bradypus*) a polylobular, and aard vak (*Orycteropus*) a zonary and deciduate placenta. Moreover, in the Carnivora (Fig. 15. 12) nearly all gradations exist between zonary (dog, cat), to a single disc in the brown bear (Subba Rau, 1925).

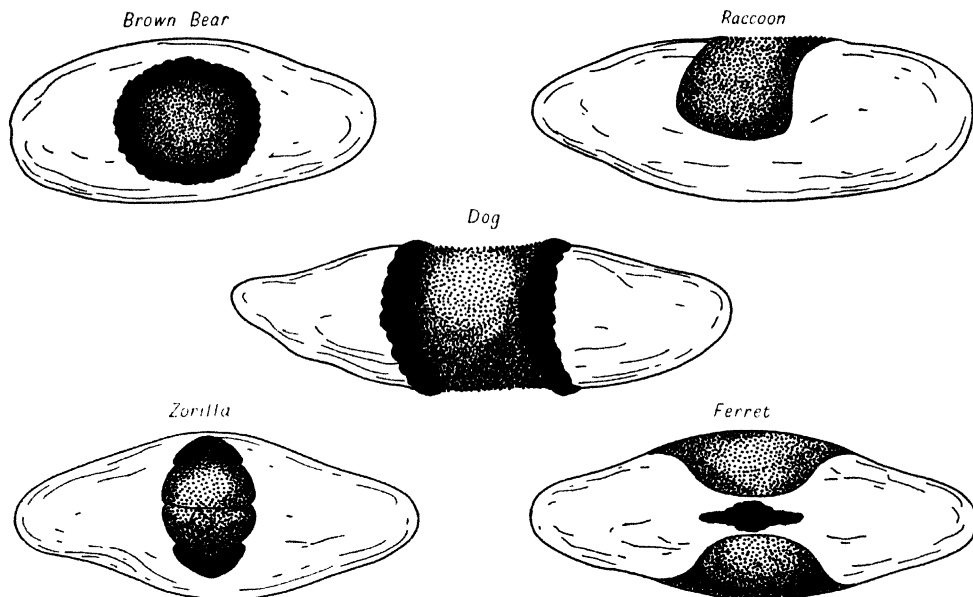


Fig. 15. 12 -- Diagrammatic representation of the placenta of the dog, brown bear, racoon, Zorilla and ferret. The diagram shows the extent of the placental band and the position of the haematoma. (Redrawn from Rau.)

Endometrial relations of the placenta

The degree of union between the foetal villi and the uterine mucous membrane varies a good deal and may involve merely the close apposition of these two tissues — *apposed placenta*, or their intimate fusion — *conjoined placenta* (V. Baer, 1828 ; Robinson, 1904). In the former, where there is no intimate fusion of the tissues, separation is easily effected at parturition without the simultaneous removal of maternal tissue and the placenta is said to be *non-deciduate*. The *deciduate* type forms an intimate connection with the wall of the uterus and at birth a variable amount of maternal tissue separates off and is shed with the placenta. The placenta of the mole (*Talpa*), the bandicoot (*Perameles*) and other marsupials ¹ is not shed at birth, but becomes gradually absorbed by the mother. For such Hubrecht (1894) suggested the term *contra-deciduate*. In the dugong (Turner, 1889) and the sheep (Assheton, 1906) part of the foetal tissue remains attached to the uterus and is absorbed by the leucocytes of the mother.

¹ Völker (1922) claims that the placenta of *Spermophilus* is never shed although Mossman and Weisfeldt (1939) state complete separation of the placenta is effected at birth.

Strahl (1906a) employing criteria suggested by Milne Edwards (1844, 1868), divides Mammals into two groups, one having a "Halbplacenta" or "Semi-placenta" and the other a "Voll placenta" or "Placenta Vera." In the former no maternal vessels are opened and the connection is less intimate, while in the latter haemorrhages occur during pregnancy. But in a physiological sense, the *half* placenta is certainly as efficient an organ of nutrition as the *whole* placenta.

Whereas Robinson emphasises the methods of attachment of the trophoblast to the uterus, Assheton (1906) lays stress on the anatomical condition of the trophoblast at the time of its first attachment. According to the latter, we have on the one hand in the Ungulata, Sirenia, Cetacea, and Nomarthra a great lateral expansion of the trophoblast, leading to the "plicate" type of placenta, and on the other hand in Insectivora, Chiroptera, and Primates cell proliferation of the trophoblast producing thickenings thereof and leading to the "cumulate" type of placenta. The Carnivora are regarded by Assheton as a central group with respect to placentation from which either cumulate or plicate type could be evolved. The Lemurs are described as "pseudo-plicate."

The differences between the two types are summarised by Assheton as follows:—

Cumulate Placenta	Plicate Placenta
Great radial proliferation of trophoblast, leading to thickening of the membrane.	Great tangential proliferation of trophoblast, leading to folding of the membrane.
Greater destruction of maternal tissue.	Lesser destruction of maternal tissue.
Much bleeding of mother.	Little bleeding of mother.
Lacunisation of trophoblast.	No lacunisation of trophoblast.
Secretion of uterine glands of less use, or in some cases of no use to the foetus.	Secretion of uterine glands of prime importance.
Degeneration of uterine epithelium and glands severe.	Little or no degeneration of uterine epithelium and glands.
Embryo or embryos seldom fill the whole cavity of the uterus.	Embryo or embryos usually fill the whole cavity of the uterus.

According to Assheton the reason for the causation of the latter character is that "fixation is brought about or other intimate connection in the cumulate type, while fixation in the plicate type depends more upon the internal hydrostatic pressure of the blastocyst up against the uterus—much as a pneumatic tyre retains its position in the iron rim of a bicycle wheel."

Classification by finer structure

In the foregoing classifications it is the morphological features and the relations established at term between the maternal and foetal tissues that have been emphasised. As the placenta is, however, essentially an organ of nutrition, and as it is the maternal blood that contains the nutritive materials required for the metabolism of the foetus, a classification based on the extent of the approximation of the maternal and foetal blood streams in the functional parts of the placenta, that is, on the number of membranes or cell layers which intervene between the two circulations, would appear to be the most logical; and from a purely functional

point of view is, perhaps, the only mode of treatment. Such a classification is that put forward by Grosser (1909 ; 1927) and later (1937) slightly modified by Mossman.

Grosser calls attention to the fact that the degree of intimacy in the association of the maternal and foetal blood is unequally established in the uteri of different mammals and rightly maintains that the " placental membrane " is the structure of greatest functional significance in any type of placenta. In the simplest forms, an intact mucosa is opposed to the chorion and the maternal and foetal bloods are separated by maternal endothelium, maternal connective tissue, maternal uterine epithelium, foetal (chorionic) epithelium, connective tissue and endothelium. In the more complex types, however, these layers, especially the maternal constituents are successively broken down, until the chorionic epithelium, which according to Grosser is always preserved intact, although perhaps modified, comes into direct contact with the maternal blood. Consequently by utilising the name of the maternal tissue which is contiguous with the chorion as the denominator, he distinguishes the following four types of placentae :

(1) *The epitheliochorial*, in which all the maternal tissues are preserved intact and the chorionic epithelium without undergoing special differentiation comes in direct contact with the maternal uterine epithelium. The gross shape of the placenta is diffuse and the endometrial relation non-deciduate. The classical example of this type is the placenta of the pig, which thus interposes between the two bloods no fewer than six membranes together with the potential lumen of the uterus with its contained secretions. This type of placenta is also met with in the horse, tapir, hippopotamus, donkey, whale, camel, pangolin, American mole, etc., and in the Lemurs among the Primates.

(2) *The syndesmochorial*, in which the uterine epithelium disappears to a greater or less extent, leaving the chorion in contact with the connective tissue of the uterus ; between the two bloods, therefore, there exist only five membranes. The external form of the placenta is cotyledonary, and the endometrial relation transitional between non-deciduate and deciduate, with contradeciduate areas in some species (sheep). Placentae of this type are found in Ruminants, though some investigators are inclined to think that the bovine placenta is actually more like the epitheliochorial type than Grosser's (1909) table would indicate. (See p. 201.)

(3) *The endotheliochorial*, in which the uterine mucosa is still further reduced and the chorionic epithelium comes in contact with the endothelium of the maternal capillaries. The membrane separating the maternal and foetal blood thus consists of maternal and foetal endothelium, strands of foetal mesenchyme and the chorionic epithelium which is often differentiated into cellular and syncytial trophoblast. An endotheliochorial placenta is characteristic of the Carnivora, and is also present in some Insectivora such as *Sorex* (shrew), *Talpa* (mole) and *Tupaia* (tree-shrew), some bats (Chiroptera), and in the Sloths among the Edentata.

(4) *The haemochorial*, in which the maternal endothelium also disappears and maternal blood directly reaches the chorionic epithelium, so that the foetal blood is separated from that of the mother by only the tissues of the chorionic villi ; that is, foetal trophoblast, connective tissue and endothelium. Typical examples of this type of placenta are found in the lower rodents, hedgehog, monkeys and man.

Grosser found it convenient to speak of a *labyrinthine haemochorial* type, such as is found in most rodents, in contradistinction to a *villous* variety such as occurs in

TABLE I.—TISSUES SEPARAT

CLASSIFICATION				MATERNAL TISSUE (Uterine mucous membrane).		
Robinson.	Assheton.	Strahl.	Grosser.	Endo- thelium.	Connective Tissue.	Epi- thelium.
Apposed Placenta	Plicate Placenta	Semi- Placenta	Epithelio- chorial			+
			Syndesmo- chorial			
Conjoined Placenta	Cumulate Placenta	Placenta Vera	Endothelio- chorial		---	
			Haemo- chorial		---	
			Haemo- Endothelial (Mossman) ³			

¹ According to Goldstein (1926) the pig's placenta should be placed in a separate Epithelio-endothelial class.

² According to Branca (1927) the bat's placenta represents an intermediate type between the definitive endotheliochorial and haemochorial placentae.

FOETAL AND MATERNAL BLOOD

FOETAL TISSUE
(Allanto-chorion).

Tropho- blast.	Connective Tissue.	Endo- thelium.	Gross Shape.	Familiar Examples.	Main Zoological Groups.	Relation to Endometrium (Huxley's classification).
	+		Diffuse	Pig, ¹ Horse, Donkey	Bunodonta, Perissodactyla, Cetacea, <i>Manidae</i> , Lemuroidea, <i>Scalopus</i> <i>aquaticus</i> .	Non-deciduate
			Cotyle- donary or mul- tiplex	Sheep, Goat, Cow	Pecora	Transitional
			Zonary to Discoid	Cat, Dog, Ferret	Carnivora, <i>Bradypodidae</i> , <i>Tupaiidae</i> , <i>Talpidae</i> , <i>Soricidae</i> , Megachiroptera ²	Deciduate (some Contr deciduate, e.g. <i>Talpa</i>)
	+		Discoid, double discoid or zonary	Man, Monkey	Primates, <i>Tarsiidae</i> , Sirenia, most Microchiroptera, Hyracoidea, <i>Myrmeco- phagidae</i> , <i>Dasypodidae</i> , most Insectivora, Lower Rodentia	
			Discoid, cup- shaped or sphe- roidal	Guinea- pig, Rat, Rabbit	Higher Rodentia (Hystri- comorpha), Lagomorpha	

³ An Haemomesothelial class is not included, since the foetal connective tissue is very sparse or even absent in the region of the foetal capillaries in all placentae, even epitheliochorial or syndesmo-chorial ones.

man. In both types the maternal blood circulates in spaces which lie exclusively in the trophoblast, but whereas in the former these spaces are narrow and capillary-like and the trophoblastic lamellae are separated by mesoderm carrying foetal capillaries, in the latter the maternal blood spaces are large and sinus-like and the villi carrying the foetal capillaries project directly into a bowl, as it were, of maternal blood. In the Primates all gradations exist from the primitive haemochorial labyrinthine placenta to the specialised haemochorial villous types (Wislocki, 1929; Hill, 1932).

Before leaving this aspect of Grosser's classification, the work of Mossman (1926) must be mentioned. In connection with his investigations of the placenta of the rabbit, Mossman pointed out the incompleteness of Grosser's (1909) classification on the basis of the tissues forming the separating membrane between foetal and maternal blood. He rejected Grosser's view that it is an "unbroken

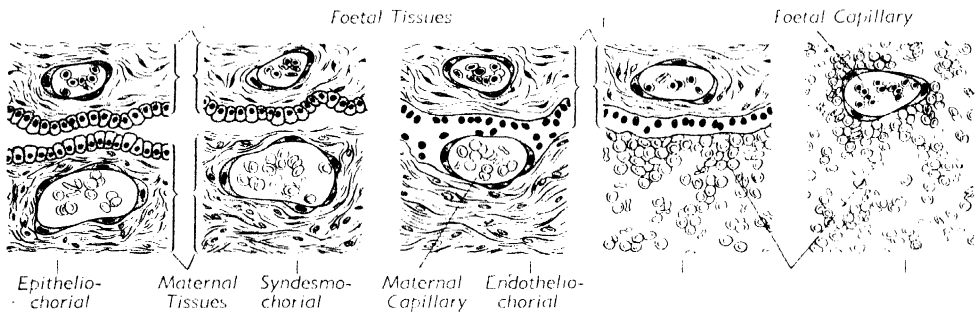


Fig. 15. 13—Histological types of placenta arranged to emphasise the progressive breaking down of the barrier between the maternal and foetal circulations. (Redrawn and slightly modified from Arey after Flexner and Gellhorn.)

rule in placentation (at least in real placentalia) that the epithelium of the chorion is, although perhaps modified, always and without exception preserved." In particular, he calls attention to the fact, already demonstrated by Duval (1890) and Chipman (1903), that the membrane between the two blood streams in the late rabbit placenta consists to a great extent of only the foetal endothelium. Accordingly, he suggested that the rabbit, rat, guinea-pig and other rodents, having the same placental structure be classified in a separate *haemoendothelial* group rather than in the haemochorial group.

Table I on pp. 148–49, compiled from several sources, and Fig. 15. 13 present the salient features of placental classification.

When considering Table I and Fig. 15. 13, one must be careful not to acquire ideas too far from reality by taking them in too formal or narrow a sense; like most other schematised diagrams they must be allowed a certain flexibility. It must be remembered that, as Mossman (1926) points out, all placentae at the beginning of their development are of the epitheliochorial type, and that the ones with less separating tissue change in construction during embryonic development. "There are," he states, "probably no placentae of the two or three thinner membraned sort which, even when fully formed, do not possess some areas of separating membrane similar to that of the less intimate types." An extreme example is

the case of the rabbit whose fully formed placenta is of the haemoendothelial type but which actually passes through stages in its development which represent almost exactly the intervening types in Grosser's classification (see Table II).

TABLE II
TISSUES SEPARATING FOETAL AND MATERNAL BLOOD IN THE RABBIT PLACENTA
AT VARIOUS AGES

Age in days	8	9	9½	10	12	17	22-Term
Maternal Tissue :							
Endothelium	+						
Connective	+						
Epithelium	+						
Foetal Tissue :							
Cellular							
Trophoblast	+						
Syncytial							
Trophoblast	—						
Mesenchyme	+						
Endothelium	+				+		
Placenta Type	Epithelio- Chorial	Syndesmo- Chorial	Endo- thelio- Chorial	Haemo- Chorial	Haemo- Meso- thelial	Haemo- Endo- thelial	

Table II certainly shows that the change from the haemochorial to the haemoendothelial condition is a gradual one. It is also apparently true that the haemoendothelial condition is reached earliest in development in those placentae which have the character best developed. The histological picture is, however, not established uniformly : for example, in the rabbit a foetal capillary may approach a trophoblastic tubule ¹ more and more closely until the trophoblast is apparently squeezed from between their adjacent portions. The trophoblast may, in fact usually does, persist at the sides of the area of closest contact. As to the foetal connective tissue, this is largely lacking and at term only very occasional argyrophil fibres remain in the labyrinth around the smallest vessels (Wislocki, Deane and Dempsey, 1946).

Finally, attention must be called to the fact that there are individual types of chorio-allantoic placentae which combine different morphological characters. For instance, in their possession of central and marginal haematoma, many of the Carnivora have, in addition to an endotheliochorial labyrinthine placenta, what is essentially a supplementary haemochorial region. The only feature about this relationship which is not typical of an haemochorial placenta is that the maternal

¹ These refer to the small maternal blood channels of the syncytiotrophoblast which lead the blood back to the maternal placenta.

blood spaces actually lie between the uterine epithelium and the chorion, instead of in spaces lined entirely by trophoblast (see p. 243). In some Chiroptera, e.g. *Miniopterus schreibersii* small marginal endotheliochorial areas supplement the main haemochorial placenta; in others, e.g. *Myotis lucifugus lucifugus* as also in the insectivore *Centetes*, it is a villous syndesmochorial area which is present as the supplementary structure. A somewhat comparable situation occurs in the placenta of the mare where syndesmochorial areas exist along with the main epitheliochorial placenta. So too in *Galago demidoffi*, where Gerard (1932) maintains that an endotheliochorial region supplements a villous epitheliochorial condition.

Histotrophe and Haemotrophe

The nutritive materials supplied to the foetus may reach it direct from the circulating maternal blood in the placenta, "haemotrophe" or through the absorption of products of the endometrium itself, "histotrophe" (Grosser, 1927). The *histotrophe* may be of two kinds: (1) *histopoietic materials*; i.e. uterine secretions elaborated or stored in the endometrium, and (2) *histolytic materials*, i.e. detritus derived from the breaking down of degenerated endometrial tissue. For all the available material supplied to the embryo Meyer (1925) has proposed the term "embryotrophe." As originally employed by Bonnet (1899), this term included only those substances which could be regarded as products of the disintegration of the maternal tissues or as glandular secretions of the endometrium, i.e. material which could be identified by morphological as distinguished from physiological methods. In the rest of this section the term will be used in Meyer's sense. Table III compiled from studies of Needham (1931) and Windle (1940) summarise the essential points.

TABLE III

Embryotrophe

Histotrophe (paraplacental)		Haemotrophe (placental)		
Histopoietic materials i.e. secreted constituents of the uterine milk.	Histolytic materials such as disintegrating endometrial tissues and extravasated blood.	Diffusible substances such as crystalloids and blood gases requiring no resynthesis.	Diffusible nitrogenous substances of high molecular weight which require resynthesis.	Absorbable substances such as most colloids and fat which probably require resynthesis by the placental barrier.

Now, as Grosser (1927) pointed out, there is a surprisingly close correlation between the means by which nutriment is supplied to the foetus and the degree of association between the foetal and maternal blood. Thus, in those animals which, like the pig, horse, sheep and goat, have placentae of the epitheliochorial and syndesmochorial types the uterine fluids (*histotrophe*) which bathe the surface of the chorion furnish a considerable part of the food supply of the embryo throughout gestation. The secretion of the uterine glands is rich in fatty substances (in ruminants, cow, sheep, etc., but not in the pig), and has the appearance of a milky liquid—hence the name "uterine milk." As would naturally be expected, the

extremest manifestation of the mainly histotrophic type of nutrition is found in the early stages of development of mammals when the association between mother and offspring is slight, or when, as in the Marsupialia, the characteristics of the more advanced types of placental circulatory systems are not developed.¹ As the approximation of the foetal and maternal blood stream becomes more intimate, histotrophe becomes largely replaced by haemotrophe. However, in mammals with endotheliochorial placentae, the histotrophe still plays a very con-

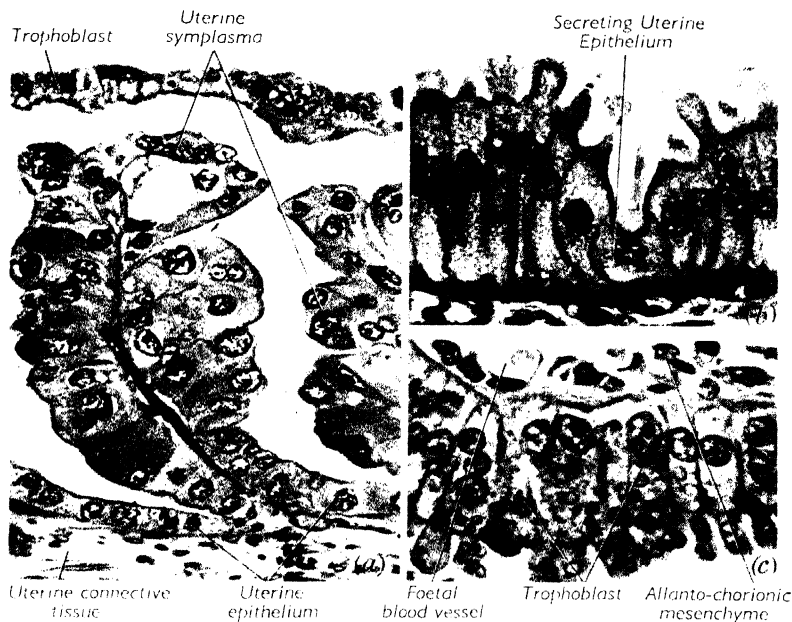


Fig. 15.14—Histology of the paraplacental region in the ferret, dog and cat. (a) Foetal and maternal tissues in the paraplacental region of the ferret, showing *symplasma* formation. $\times 235$. (b) Columnar uterine epithelium from opposite the paraplacental chorion in the dog, showing cells discharging secretion. $\times 480$. (c) Ingestion of extravasated maternal blood corpuscles by the trophoblast in the cat. $\times 550$.

siderable rôle. In the Carnivora, for example, the uterine glands are conspicuous and together with the uterine epithelium produce a secretion of value to the foetus (Fig. 15.14). Moreover, the haematoma represented by the "green" and "brown" borders in the dog and cat and consisting of large blood extravasations between the chorion and endometrium have been assigned a nutritive rôle, particularly in the transfer of iron. Histotrophe is also supplied to the foetus by the widespread absorption of uterine symplasma.² In the haemochorial and haemoendothelial placentae of man and the higher rodents, on the other hand, histotrophic nutrition

¹ In the most successful ovoviviparous sharks (e.g. *Trygon*) it would appear "that the nourishment of the embryo by means of the clasmobranch *histotrophe* is so efficient as even to surpass the efficiency of the viviparous types (e.g. *Mustelus laevis*) in which the yolk-sac actually invades the wall of the uterus" (Needham, 1942).

² Symplasma in placental terminology refers to the fusion of maternal cells.

is insignificant after the early stages of development and nourishment of the foetus becomes possible largely by direct absorption from the maternal blood.

Inter-relations of the mesonephros, metanephros and the placenta

Other variations in pre-natal functions have been related to differences in the development of the foetal membranes. The placenta, in addition to its nutritive rôle, serves also for the removal of waste products. Those animals (e.g. pig, horse, sheep, etc.), therefore, which interpose a substantial barrier between the maternal and foetal bloods might be expected to have more difficulty in eliminating waste into the maternal circulation than those like the rabbit, guinea-pig, rat and man in which the placental membrane is thin and the placental excretory route is open. In the first group, as is well known, the allantois is an enormous vesicle and apart from vascularising the placenta acts as a receptacle for the foetal urine. In the second group, on the other hand, the endodermal portion of the allantois is rudimentary or entirely absent and therefore of little consequence.

From these and other considerations, Bremer (1916) has attempted to correlate the variations in the size of the allantois with the degree of intimacy between maternal and foetal bloods, and with the size and activity of the mesonephros. Thus, according to Bremer, those species with large allantoic vesicles have large functional Wolffian bodies and inadequate provision for early placental elimination; whereas in those in which the allantois is small, mesonephric activity is slight and elimination of waste takes place mostly or wholly through the placenta. Bremer also tried to correlate the appearance of the thin, membranous trophoblastic plates in haemochorial placentae with the functional failure of the mesonephros. He assumed, however, that urinary excretion ceases at the time that the mesonephros has degenerated. But since it has been shown that the mesonephros and metanephros can, and probably do, function simultaneously and continuously, the comparisons attempted by Bremer have been questioned (Gersh, 1937). Nevertheless, the experimental results showing continuous urine formation do not imply that the thinned-out syncytial plates of the chorionic villi do not serve as areas through which foetal waste products are excreted. But as Gersh put it, commenting on Bremer's results: "they destroy the so-to-speak causal relationship established by Bremer between the appearance and frequency of the placental plates and the functional failure of the mesonephros." For a further discussion see also the papers of Cunningham (1923), and Wislocki and Bennett (1943).

The Decidua

General nature and occurrence. The conversion of the uterine mucosa, already considerably altered in the pre-ovulatory period, proceeds rapidly after the fertilisation of the ovum and soon leads to a great increase in thickness of the gravid endometrium. At a certain stage it reaches its full development, and then gradually diminishes till, at the end of gestation, it forms only a thin layer (e.g. many rodents and man) through which separation of the placenta is normally effected at term. The mucosa of the pregnant uterus is, therefore, called the *decidua*, a name which was first used to designate the maternal tissue which is shed with the after-birth at parturition.

For purposes of description the decidua is divided into three regions on the basis of its relations to the chorion : (1) the *decidua capsularis* or *reflexa* (absent in those forms with central implantation), which overlies the ovum and separates it from the rest of the uterine cavity ; (2) the *decidua basalis* or *serotina*, which underlies the ovum and forms the maternal component of the placenta and, (3) the *decidua vera* or *parietalis* which forms the rest of the endometrium.

In the uterine mucosa during pregnancy the most noticeable change occurs in the interglandular tissue of placentae of the typically cumulate or deciduate varieties in which *decidual cells* are formed. Our principal conceptions (and misconceptions) of the exact nature of this tissue have been derived largely from what has been learnt of its nature in the human and rabbit placenta, and in what follows these species will, in large measure, be utilised for purposes of interpretation of this part of the subject of placentation.

Decidual cells vary a good deal in their structure, morphological rank, and mode of origin, but it is nevertheless useful to have before us the idea of a " typical " decidual cell, as an endometrial connective tissue cell which has enlarged and become rounded or polyhedral due to the accumulation of glycogen or lipoids within its cytoplasm. This is obviously a crude description, rather than an adequate definition, but it will serve our immediate purpose.

It is important, at the outset, to bear in mind that the process of decidual cell formation varies widely both in rate and degree in different species. This is strikingly true of the Artiodactyla, Carnivora, Rodentia and Primates, especially in those forms with interstitial implantation. Certainly as regards decidual transformation the cellular changes in the Carnivora are moderate as compared with Rodents, but are even more so in the Ungulata, amongst which there are several species (e.g. pig, cow, sheep and goat), in which it is even doubtful whether there are any cells which should be considered decidual.¹ Here, however, one should guard against the common error of supposing that the formation of decidual cells is directly determined by the presence of deciduating maternal tissues. There is little evidence in support of this, while it is opposed by the fact, later to be described, that in such forms as the horse (see p. 183) and in contra-deciduate species (e.g. *Talpa europaea*) practically no maternal tissue is shed at parturition, yet there is a distinct transformation of the endometrial connective tissue, the cells of which acquire the characteristics of true decidual cells such as are found in the placentae of man or of any of the other typically deciduate species.

The decidual cells of different species of mammals exhibit quite marked differences of size, form and structure. In man, for example, two kinds of decidual cells are regularly present, large and small (Marchand, 1904). In their most typical form they are large, oval or, from mutual pressure, polygonal cells with an epithelioid appearance and frequently attain a diameter of 0.05 mm. or more. The majority have one large, spherical nucleus although binucleated cells are not uncommon ; they are fully differentiated and show, at the most, only amitotic divisions. Physiologically they are localised areas of specific chemical transformation, producing characteristic products, such as glycogen, lipoids, pigments of various kinds and perhaps hormones (e.g. mare) and may be classified accordingly. In their least differentiated condition they are small round cells

¹ For further details, see Wislocki's (1941) account of the placenta of *Mudoqua (Rhynchoniragus) kirkii*, also Mossman (1937).

whose bodies are almost filled by the nucleus ; these latter are present at all stages of pregnancy, but are most abundant during the early stages. According to Marchand, they appear to be the forerunners of new decidual cells.

In his monograph (which should be consulted for the special literature), Mossman (1937) gives details of the conditions as they are observed in the Rodentia and Lagomorpha. In the domestic rabbit (*Oryctolagus cuniculus*) at least three cell types are involved : (1) *uninucleate* decidual cells which appear early in pregnancy as *perivascular sheaths* (Masquelin and Swaen, 1879), (2) much larger vesicular *multinucleate* cells, derived apparently from the uninucleate ones (Maximow, 1898 ; 1900 ; Chipman, 1903) and, (3) the so-called "*myometrial gland*" cells which are found in the myometrium of the subplacental region from about the twenty-second day of pregnancy until term (Ancel and Bouin, 1911). In the latter the connective tissue cells become enlarged and swollen in a similar way to those of the mucosa which give rise to decidual cells, but to a less extent. According to Mossman (loc. cit.), these cells are especially well developed in the placentae of *Lepus americanus* (snowshoe rabbit) and *Sylvilagus floridanus* (cottontail rabbit), while in the placentae of *Ondatra zibethica* (musk rat), *Perognathus* (pocket-mouse), *Dipodomys* (kangaroo-rat) and *Geomys* (pocket-gopher), he has traced their origin back through transitional stages to small round cells infiltrating the basal myometrium and adjacent mesometrium. For an account of decidual cells in the rat, see Krehbiel (1937).

Origin of decidual cells. Various opinions have been held regarding the origin of decidual cells. According to some investigators they are modified epithelial cells (mouse, Frommel, 1883 ; man, Overlach, 1885), or endothelial cells (man, Ercolani, 1876 ; Wade and Watson, 1908 ; water vole, Sansom, 1922 ; and others), or enlarged and modified leucocytes (man, Henning, 1872 ; Langhans, 1877). But the most convincing evidence shows that the majority arise, as Creighton (1878) first suggested for the guinea-pig, from undifferentiated, perivascular mesenchymal cells which, (in the presence of a favourable endocrine situation) enlarge and accumulate stores of glycogen or lipid within their cytoplasm. Masquelin and Swaen (1879) demonstrated this mode of origin in the rabbit and were supported by Minot (1889), Chipman (1903), Mossman (1926) and others. The observations of Hart and Gulland (1892), Peters (1899) and Leopold (1906) on early embryos showed that the same origin was most probable in man, and this view has been upheld by many recent investigators (see Bryce and Teacher, 1908 ; Grosser, 1927 ; Mossman, 1937 ; Baker, Hook and Severinghaus, 1944 ; and Wislocki and Dempsey, 1945). The connective tissue origin has been further confirmed by the study of so-called ectopic decidua, in which typical decidual cells develop from the connective tissues in situations far from the uterine cavity¹ (Williams, 1905 ; Geipel, 1928).

The first appearance of decidual cells in the superficial layers of the mucosa has suggested a stimulus for their formation arising from the product of conception (Fraenkel, 1903). However, starting with the initial observations of Loeb (1908)

¹ Large cells proliferating into the lumen of the vessels, especially the arteries near or in the placenta in various mammals, have been considered by different investigators as arising from the trophoblast (man, Grosser, 1927 ; rabbit, Mossman, 1937), endothelium (rabbit, Maximow, 1898 ; Mossman, 1937 ; rat, Goldmann, 1912 ; macaque, Hill, 1932 ; Wislocki and Streeter, 1938), muscle cells (*Centetes*, Goetz, 1937).

in the guinea-pig, it has been shown by a number of investigators (reviewed by Selye and McKeown, 1935; also Wislocki and Streeter, 1938) that the development of deciduomata in several species of mammals is independent of a foetus and its trophoblast, since it can be induced experimentally in the non-gravid uterus by mechanical (Loeb, 1908; Long and Evans, 1922) or electrical (Krehbiel, 1937) stimulation of the endometrium. This decidual reaction depends directly upon the presence of functional corpora lutea and will not develop in response to trauma, except during the brief interval which corresponds to the time when the ovum normally becomes attached to the uterine mucosa (Loeb, 1908; Hammond, 1917; Long and Evans, 1922; Parkes, 1929; Courrier and Kehl, 1930; Brouha, 1934 and others).

Reference has been made in an earlier paragraph to the origin, from small round cells, of the myometrial gland cells found in certain rodents. According to Wederhake (1906), plasma cells also occur in the decidua, and transitions between these and typical decidual cells. Similar evidence is also afforded in the case of the horse, by the relationship which undoubtedly exists between small round cells (lymphocytes), plasma cells, and the interglandular cells of the "endometrial cups" (see p. 184). We have therefore to recognise that in the production of decidual reactions in certain mammalian species, the undifferentiated mesenchymal cells may pass through a transitory state in which they cannot be distinguished from lymphocytes. However, until the facts have been decisively demonstrated by the study of living cells judgment on these cases should be suspended. Nevertheless, the foregoing observations prominently raise the question whether lymphocytes may not play (as they do in inflammation) a more active and important part in decidual reactions than most writers have hitherto assumed.

Individual decidual cells have probably a short life history. Even at a comparatively early period many of them are found in various stages of hyaline degeneration, giving rise in part to layers of fibrin, and as pregnancy advances there is a gradual extension of the fibrinous change. The degeneration of the decidual tissue would seem to be due to the influence of the foetal ectoderm, as in man it occurs much earlier and more abundantly in the basalis and capsularis than in the vera (Webster, 1901). Its gradual diminution during pregnancy indicates an absorption of the decidua and it is now generally accepted that the foetal ectoderm from the earliest stages of pregnancy is able to disintegrate the cells with which it comes in contact, and to absorb the degenerate products.¹ However, there must be a certain amount of resorption by the maternal organism, for in contra-deciduate species (e.g. *Talpa*) the placenta does not separate in the third stage of labour, but remains *in utero* and is resorbed.

Functions of the decidua. The physiological meaning of the decidual cells still remains one of the most obscure questions of placentation. There are some who maintain that decidual cells are, from the beginning, degeneration forms of the connective tissue cells and are of use only as pabulum to be absorbed by the ovum. But during the whole of pregnancy, there exist in the placenta of some species decidual cells which, in their appearance and staining properties, show no resemblance to degenerated cells. From their abundance and great specialisation they have in all likelihood definite functions to perform but their full significance is

¹ To that part of the foetal ectoderm which is thus adapted for the acquirement of embryonic nutriment the name *trophoblast* has been given by Hubrecht.

not understood. Their first formation dates from the destruction of the surface epithelium when the blastocyst comes in contact with the connective tissue, and the earliest to appear are in the neighbourhood of the ovum. Their position and general appearance in different orders suggested to Turner (1876a) and others a maternal reaction against the advance of the parasitic ovum. Fothergill (1899) speaks of the decidua preventing the injurious invasion of the uterine wall by the foetal elements and Hammond (1917) concluded that the maternal placenta serves at first as a source of foetal nutrition and at all times provides against the too intensive penetration of the ovum, thereby preventing permanent attachment of the embryo itself to the mother. Chipman's (1903) figures of the placenta of the rabbit show that the trophoblastic columns (tubules) advance more rapidly where they encounter a vessel than where they lie against decidual cells. Wade and Watson (1908) have noted their resemblance to young granulation tissue in the mucosa of the Fallopian tube in an early ectopic pregnancy. Bryce and Teacher (1908), in their description of an early human ovum, say: "The decidua formation is a process of a conservative nature, by which, during the early months of pregnancy, the activities of the trophoblast are limited and controlled until such time as placentation is complete." Whether or not the decidua forms a protection to the mother, there is increasing evidence that the trophoblast does not invade the decidua to the extent supposed by the older authorities. This was first emphasised by Hubrecht (1889) in the hedgehog, and later by Bryce and Teacher (1908), and has more recently been advocated by Mossman (1937) and Wislocki and Streeter (1938).

According to a widespread belief the decidual tissue forms a cleavage zone at the time of parturition, and also helps prevent excessive haemorrhage from opened blood vessels in the placental area (Mall, 1915). This, however, is purely hypothetical. Certainly as regards decidual transformation the cellular changes in the cat are moderate as compared with the rabbit, yet the former has no more difficulty in cleaving during the third stage of labour than the latter. Moreover, in the contra-deciduate mole (*Talpa*) the placenta does not separate at term although there is still a well-developed decidua present (Mossman).

At one time discussion centred about the question whether or not decidual cells assumed endocrine functions. Nattan-Larrier (1900) was among the first to regard them, as somehow concerned in the elaboration of an internal secretion. Later Ancel and Bouin (1911) from a study of the changes occurring in the mammary glands of the pregnant rabbit, concluded that the glandular development which takes place in the last part of pregnancy is caused by the cells of the "myometrial gland." Hammond (1917) occasionally found these cells in the rabbit's uterus, but their occurrence, he considered, was too irregular and uncertain for them to have the important endocrine functions which their discoverers attributed to them. This is also the conclusion of Mossman (1937), who states that "one is absolutely unjustified, from anything yet known of their cytology, in making assumptions of endocrine functions for them." However, observations have begun to accumulate in favour of the conclusion that the decidua may be concerned in the secretory processes of the placenta. An interesting example of this is seen in the development of the so-called "endometrial cups" in the uterus of the pregnant mare. The endometrial cups are specialised structures to which one can attribute special secretory activity, and in which a decidual transformation of

the interglandular tissue has been described by a number of workers (Cole and Goss, 1943 ; Amoroso, 1945 ; Rowlands, 1947), all of whom believe they have traced to these cups an important source of equine gonadotropin.

In Rodents the decidual cells have an important and definite part to play in synthesising and storing glycogen as a supply of carbohydrate for the foetus (mouse, Jenkinson, 1902 ; Driessen, 1908 ; rabbit, Chipman, 1903 ; Lochhead and Cramer, 1908 ; Loveland, Maurer and Snyder, 1931). In man also, the decidual cells contain glycogen at an early period (Driessen, 1907 ; Froboese, 1924 ; Dempsey and Wislocki, 1944, and others). Fat globules infiltrate the decidual cells of various animals (rabbit, Chipman, 1903 ; Gilbert, 1942 ; horse, personal findings ; man, Gillman, 1941 ; Wislocki and Dempsey, 1945) at a stage when there is no question of a fatty *degeneration* taking place in the cells. On the other hand, the occurrence of lipoid droplets in the giant cells of the lamellae of the late cat placenta has been regarded as a manifestation of senescence (Wislocki and Dempsey, 1946a). For a discussion of the significance of glycogen and lipides in the placenta, see Chapter 16 and also Wislocki, Deane and Dempsey (1946). Finally, the cells appear to have the power of ingesting and decomposing erythrocytes, but the relations to the iron-metabolism of the foetus require further study. For an account of "giant cells," see p. 261 and Mossman (1937).

VI.—PLACENTAL PHENOMENA IN MARSUPIALS

The initial steps taken by mammals to compensate for a reduced yolk supply seem to have been (1) the specialisation of the lining membrane of the uterus as a food supplying mechanism ; (2) the utilisation of the yolk-sac for absorbing secretions and other nutritive substances present in the *uterine milk*. In all Marsupials the yolk-sac is very large whereas the allantois is always relatively small, and only in *Perameles*, *Phascolarctos* and *Phascolomis* does it vascularise a placenta. In all other marsupials so far investigated the embryo is nourished exclusively through the yolk-sac and a definite yolk-sac placenta of somewhat complex character is present. For details of marsupial placentation it will be sufficient to refer here to studies of Selenka (1886), Hill (1918) and Hartman (1920) on *Didelphys aurita* and *Didelphys Virginiana* ; Hill (1900a) on *Dasyurus viverrinus* ; and Hill (1897 ; 1900b) as well as Flynn (1922 ; 1923b ; 1930) on *Perameles*, through which the literature can be reached.

Didelphys. In the opossum the mesoderm spreads about halfway round the wall of the blastocyst, but it does not split over its whole extent. Hence the coelom is small, and correspondingly the separation of the yolk-sac and trophoblast is insignificant (Fig. 15. 15). The allantois grows out into the coelom only to impinge on and invaginate the wall of the yolk-sac. It never comes in contact with the outer wall of the blastocyst. Thus the extra-embryonic mesoderm together with the exo-coelom resemble a connecting stalk by means of which the embryo, together with the amnion and allantois, is suspended from the wall of the blastodermic vesicle and floats, as it were, in the concavity of the yolk-sac. An extensive pro-amnion is present.¹

¹ The name pro-amnion is given to that region of the amnion below the head of the embryo from which the mesoderm is absent.

In the mesoderm of the yolk-sac there is an area vasculosa, and at the extreme end an annular vessel—the *sinus terminalis*. The part of the wall where the mesoderm is unsplit is thrown into folds which fit into corresponding furrows of the mucosa. Hence an avillous yolk-sac placenta is formed (Selenka, 1886). The nutrition in the uterus is very primitive. The eggs contain a comparatively large supply of yolk granules for the initial stages of development. As they travel along the oviduct and enter the uterus, which they do in 24 hours or a little less, they are invested with a thick nutritive layer, derived from the secretion of the tubal and uterine glands. Later the embryos are also nourished by the primitive placental

structures for a period short in duration, but long enough to allow of the differentiation of their main organs and systems. In the mucosa the surface epithelium remains intact. The only change is an oedema of the layers, and the sole nutritive material is a watery fluid, composed of glandular secretion and a lymph transudate almost devoid of cells. It is absorbed by the trophoblast cells, which here and there enlarge to enormous "Nährzellen" (Selenka, 1886), similar to those found in certain *Eutheria* (see Fig. 15. 57 *b*), and so increase the absorbing surface. After about 12½ days the immature young are born (Hartman, 1921) and are then transferred

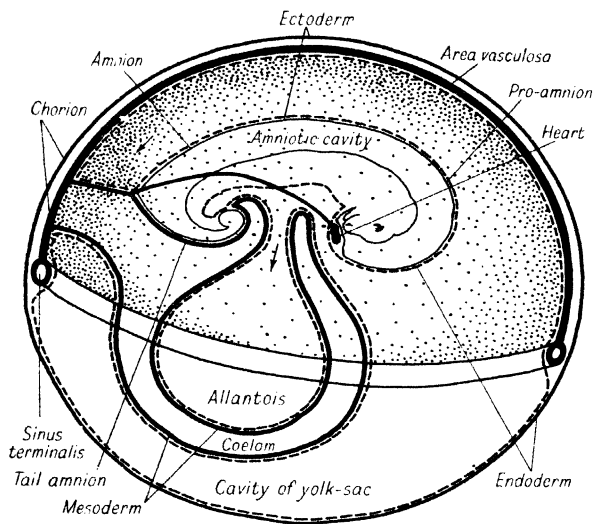


Fig. 15. 15—Diagram of an opossum embryo and its appendages (Redrawn and slightly modified after Minot.)

to the pouch and nourished by the mammary secretion. The number of young that are born ranges from 8 to 12 (Grinnell, Dixon and Linsdale, 1937); intrauterine mortality being, in some cases, as high as 50 per cent.

Dasyurus. In the native cat (*Dasyurus viverrinus*) investigated by Hill (1900a), the major half of the outer wall of the blastodermic vesicle is devoid of mesoderm and blood vessels, while over the remainder of its extent it is formed, in about equal proportions, by a discoidal area of true chorion and a relatively small zone of trilaminar omphalopleur which corresponds to the embryonic area vasculosa. The allantois is long and hand-like and destitute of any differentiated stalk. It is vascular over a small area and comes in contact, for a time, with the chorion. But, according to Hill (1900a), the allantoic vessels degenerate rapidly and completely, and the allantois again lies free in the coelom as an histologically degenerate, non-vascular, and quite vestigial structure (Fig. 15. 16).

Correlated with this degenerate and functionless condition of the allantois, a somewhat complex yolk-sac placenta associated respectively with the vascular and non-vascular portions of the omphalopleur is present; the latter being the more

important physiologically. In the region of the area vasculosa the wall of the yolk-sac adheres to the uterine epithelium, and, as in the opossum, forms a simple vascular chorio-vitelline placenta. The superficial capillaries of the mucosa, which are slightly dilated, are separated from the vitelline vessels by the uterine epithelium and a thin layer of foetal ectoderm. Through the two layers the gaseous exchange probably takes place. Beyond the sinus terminalis, the non-vascular part of the wall unites over an annular zone with the uterine epithelium by enlarged foetal ectodermal cells to form the non-vascular segment of the yolk-sac placenta (bilaminar omphalopleur). The cell outlines between the foetal trophoblastic cells very soon disappear with the consequent conversion of the layer into a syncytium. These syncytial "Nährzellen" are phagocytic, and enclose fragments of uterine epithelium and superficial capillaries. To this admixture of maternal and foetal elements, the name "diploplasma" may be applied (Flynn, 1923b). The endoderm of the yolk-sac which is in protoplasmic continuity with the ectoderm also loses its cellular character and a series of irregular clefts separated by cytoplasmic processes appear between the two layers. At the end of gestation the greatly dilated maternal capillaries rupture and the extravasated blood passes into sinus like spaces in the conjoined syncytial mass, whence it is transmitted to the cavity of the yolk-sac and serves for nutrition (Hill, 1900a). Accordingly, there are regions in his non-vascular yolk-sac placenta of *Dasyurus* in which haemochorial relations are established, for the syncytial trophoblast is bathed by maternal blood. The omphalopleur of the lower polar region is apparently of no great physiological importance in the nutrition of the embryo. The gestation period is not known for certain but probably lasts 8 to 14 days (Hill and O'Donoghue, 1913).

Bettongia. In the rat kangaroo (*Bettongia cuniculus*) the allantois remains small and does not fuse with the chorion, consequently a chorio-allantoic placenta is never established (Flynn, 1930). However, an efficient yolk-sac placenta is present involving the close apposition and, in places, actual union of the trophoblast with the uterine epithelium. This fusion is most intimate in the region of the vascular trilaminar omphalopleur and a chorio-vitelline placental relation is established. "The two blood streams, foetal and maternal are separated by the two epithelia, themselves very thin, and the endothelia of even greater tenuity. To

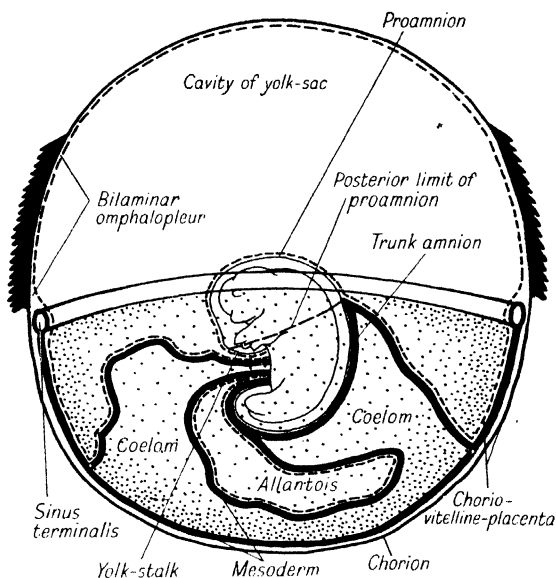


Fig 15. 16—Diagram showing the arrangement of the foetal membranes in *Dasyurus*. (Redrawn and slightly modified after Hill.)

these may be added, but not always, a small proportion of maternal connective tissue" (Flynn). Over the full extent of the vesicle, the trophoblast cells are greatly enlarged and at certain points of the bilaminar omphalopleur, between the foetal and maternal tissues, there occurs abundant "uterine milk" consisting of cell debris, leucocytes, and other material, all being actively absorbed and ingested by the foetal ectodermal cells. As in other marsupials the placenta is contra-deciduate, and at parturition the foetal membranes remain behind in the uterus to be absorbed. The period of gestation is about 6 weeks.

Perameles. In the bandicoot (*Perameles*) the placental structures are better developed (Hill, 1897, 1900b). A true chorio-allantoic placenta is present as

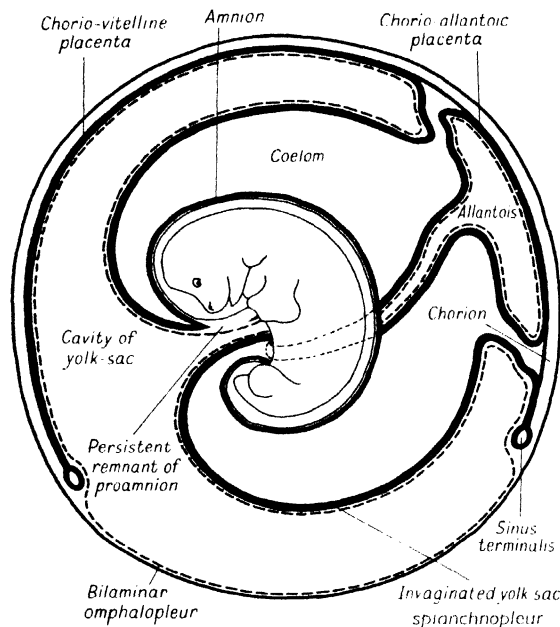


Fig. 15.17 Diagram showing the arrangement of the foetal membranes in *P. rameles*. (Redrawn from Hill.)

well as a chorio-vitelline placenta (Fig. 15.17) and there is considerable evidence of absorption in the bilaminar omphalopleur (Flynn, 1923b). Before the attachment of the blastocyst, the uterine mucosa undergoes preliminary changes. The capillaries increase in size and new vessels are formed; the interglandular tissue is composed of a loose network of anastomosing cells and the intercellular spaces are filled with lymph. The glands increase in length and diameter, and the cells of the surface epithelium lose their boundaries and fuse to form a "vascular syncytium" (Hill, 1897), analogous to the *symplasma* of higher forms. The deeper surface of the syncytium is distinctly lobulated and maternal capillaries passing up between the lobules, enter the syncytial

mass, and form a network on and just beneath its surface (Fig. 15.18 a). At the same time the syncytial nuclei proliferate and migrate to the deeper portion of the lobules where they become clumped in "nests," which according to Flynn (1923b) are more or less definitely oriented "as a layer bounding a central space" and which this author believed to be an abortive attempt at the formation of crypts such as were described by Hubrecht (1893) in the placental area of *Sorex*.

Opposite the coelom, the blastocyst becomes attached to a greatly thickened discoidal area of the uterine symplasma by means of enlarged chorionic ectodermal cells, and later its wall is vascularised by the allantois so that a functional allantoic placenta is developed. After attachment, the chorionic ectodermal cells, like the early trophoblastic proliferation in man, differentiate into a basal layer, the cytotrophoblast, and a peripheral layer, the syncytiotrophoblast (Fig. 15.18 b). At various points the syncytiotrophoblast invades the uterine epithelial symplasma

ingesting the maternal elements, particularly the nuclear nests, and passing on the histotrophic material to the cytotrophoblast, the cells of which exercise a secretory and selective rôle (Flynn, 1923b). The invasion of the maternal tissue does not extend beyond the proliferated epithelium (Fig. 15. 18c). To the conjoined layer of foetal and maternal epithelia the term "diploplasma" has been applied.

As pregnancy advances most of the cytotrophoblast layer disappears, being converted into syncytiotrophoblast, and where gaps have occurred in the disorganised cellular layer the allantoic vessels become firmly attached to the proliferated layer of the foetal trophoblast into which the maternal vessels penetrate. A regular interlocking of maternal and foetal tissues is produced and the two systems of blood vessels approach each other more and more closely until the trophoblast is apparently squeezed from between their adjacent portions; an arrangement which may be designated "endothelio-endothelialis" in accordance with Grosser's terminology (Fig. 15. 18 d).

Consideration of the facts just described leaves us with rather a different conception of the history of the foetal ectoderm in the allantoic placenta of *Perameles* than is expressed

by Hill in his paper of 1897. Hill (1932), however, gives up his former position, after a reinvestigation of the problem, and admits the truth of Hubrecht's (1908) and Flynn's (1923b) conclusions that the syncytial layer of the allantoic placenta is not purely maternal, "but is of composite origin and really formed

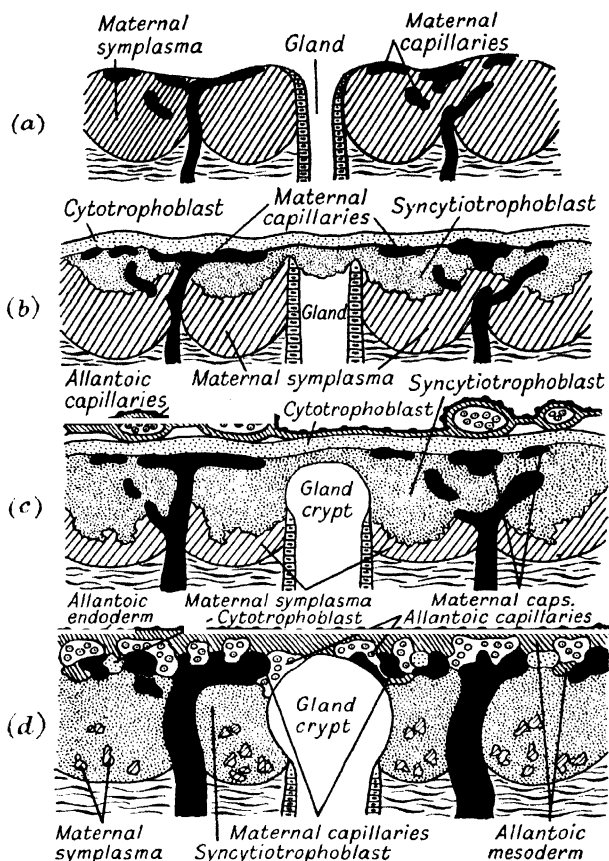


Fig. 15. 18—Diagram showing the development of the allantoic placenta in *Perameles*. (After Flynn.) (a) Formation and vascularisation of the maternal symplasma. Blood vessels and glands penetrate between its lobules and the capillaries ramify at the surface. (b) Chorionic attachment and differentiation of cyto- and plasmoditrophoblast. The gland mouths are closed and the maternal capillaries ramify just below the cytotrophoblast layer. (c) Vascularisation of the chorion and formation of foeto-maternal diploplasma. The gland epithelium has disappeared and the gland is closed in above by cytotrophoblast and plasmoditrophoblast. Capillaries still ramify below the cytotrophoblast. (d) The cytotrophoblast and maternal symplasma have disappeared for the most part and the foetal and maternal blood vessels have now come into intimate apposition. (*Placenta endothelio-endothelialis*.)

by the fusion of the allanto-chorionic trophoblast with the maternal syncytial layer which results from the proliferation of the uterine epithelium and the concomitant ingrowth of maternal capillaries."

Outside the allantoic placental site, the part corresponding to the area vasculosa is also attached by an annular zone to the uterine mucosa, and a chorio-vitelline placenta is formed. The non-vascular part of the wall is bathed by the uterine fluids as in the opossum. The uterine epithelium to which the vascular (trilaminar) omphalopleur is attached is more highly vascular than the allantoic placental syncytium, and there is little doubt that the yolk-sac placenta in *Perameles* functions as a supplementary structure before the attachment of the allantois. It is not yet determined whether it remains functional till birth. According to Hill (1897) the wall probably breaks up before the end of pregnancy, but, according to Flynn's (1923b) estimate, it may persist and function, less efficiently perhaps, until the end of pregnancy. The allantoic placenta, on the other hand, remains active, and at the time of birth (Foetus G.L. 14 mm.) the foetal portion of the placenta including portions of the allantoic stalk remain behind and are absorbed *in situ*.

VII.—PLACENTA EPITHELIOCHORIALIS

In its histological structure the epitheliochorial placenta is exceedingly simple. There is little or no destruction of the uterine mucosa, and the foetal membranes, all of which remain external to the uterine tissues, become apposed in a simple way to the surface of the intact endometrium. According to Grosser's concepts we have to deal, therefore, with a quite substantial barrier between the maternal and foetal circulations, for in addition to the epithelial components—uterine epithelium and trophoblast—the two bloods are separated by maternal endothelium, maternal connective tissue, foetal endothelium and foetal connective tissue. It is worth while to point out, however, that the extent of this theoretical barrier may be greatly reduced and that in actual fact two of these layers—the foetal and maternal connective tissue—may be practically wanting in some forms (e.g. pig, peccary, sei whale). In addition the association of the two circulations may be made still more intimate by the invasion of the foetal capillaries into the chorionic epithelium and by the occurrence of a capillary net immediately beneath the uterine epithelium.

Among the domestic mammals the pig, horse, donkey and camel are known to possess an epitheliochorial placenta. This sort of placenta is also met with in the whale (Turner, 1870, 1871, 1876c), porpoise (Wislocki, 1933a), dugong (Turner, 1889), peccary (Wislocki, 1931), tapir (Schauder, 1929), hippopotamus (Grosser, 1927), chevrotain (Strahl, 1905a), and the pangolin (de Lange, 1933), and recently has been described as the placenta type in *Scalopus aquaticus*, the American mole (Mossman, 1939a). Among the Primates, too, there are instances of this type of placenta, for all the true Lemurs are characterised by the possession of this same non-deciduate, diffuse type of placenta.

In the description which follows we shall begin with the pig as exhibiting structurally the simplest type among the Ungulata.

The Foetal Membranes and Placenta of the Pig

Gross appearance of the allanto-chorionic sac

Each allanto-chorion (Fig. 15. 11 a) with its contained embryo is a distended fusiform sac whose wrinkled surface is loosely applied to the uterine mucosa and

from which it can be readily stripped off without damage to either. The embryos are generally situated in the central portion of each sac and are usually uniformly spaced and the numbers approximately equally distributed between the two uterine horns, even when as sometimes happens, one ovary is more prolific than the other (Corner, 1921a ; Warwick, 1926). In the sow litter size differs greatly with the breed, with the age and condition of the mother, and with the order of the litter, but is usually stated to vary from 8 to 16.

Externally, constrictions of the uterine lumen mark the limits of each sac in early stages, and the swollen portion containing an embryo and its membranes is known as a "loculus" or "locular enlargement." As gestation advances, the constrictions become less conspicuous and the terminal portions of neighbouring chorionic sacs, for a distance of several centimetres, become caked together by means of a straw-coloured, glue-like deposit of the uterine milk. Adjacent chorionic sacs may sometimes fuse. Hughes (1927 ; 1929) has shown that when the foetal membranes of adjacent embryos of opposite sex fuse, the female may be modified into a free-martin. She finds, however, that impairment of gonadal development is not quite so profound as in cattle (*see* p. 206), since the germinal epithelium continues its activity. The later history of the pig free-martin is still unknown.

The allantois and the amnio-allanto-chorion. By the twenty-fourth day of pregnancy when attachment may be said to be completed, the allantois has attained a length of approximately 24 cm. and a diameter of about 5 cm. and continuing to expand with extraordinary rapidity soon occupies most of the exo-coelomic space. Meanwhile the amniotic sac, closely investing the foetus, has remained relatively small and is crowded against the chorion by the expanding allantois. Later on it expands rapidly and soon occupies a large portion of the exo-coelom, practically obliterating the central part of the latter. Where amnion and chorion are pressed together they fuse, so that at this stage the appearance is more that of a large amnio-allanto-chorionic sac with two allanto-chorionic appendages—one at each pole. These two sac-like appendages retain their communication with each other and with the urachus with which they form a characteristic T-shaped junction. The embryo meantime, enclosed in its amnion becomes oriented so that it usually lies with its left side adjacent to the amnio-chorion, its right side facing the stalk of the allantois. Essentially similar relations are found in cattle. These are well illustrated in Figs. 15. 11 *b* and 15. 45.

In advanced stages the ends of the allantoic sac become constricted and undergo a partial degeneration, (Fig. 15. 11 *a*) producing the characteristic atrophic tips—the so called "diverticula allantoidis"—which are covered by the degenerated ends of the chorion (Jenkinson, 1913 ; Heuser, 1927). According to Jenkinson these are sharply marked off from the main body by an annular thickening provided with a sphincter muscle. On the other hand, Zietzschmann (1924) and Grosser (1927), advanced the view that the allantoic tips fail to reach the ends of the chorion and Patten (1931) shows this in his Fig. 45 for embryos of 30 mm. or more. This latter condition is probably the exception rather than the rule, for it has been found in few instances in the present investigation. It would appear, therefore, that the continued differentiation of vascular channels over the allantoic tips has been very limited, and that for the later functional activity of these structures, there is required a much greater vascular richness than for the metabolic activity associated with their growth processes in early stages.

The body of the allantois is well supplied with blood vessels. Two umbilical arteries of relatively large size, coursing along its lesser curvature carry blood to it, and it is drained by veins which ultimately open into a large umbilical vein. Over its entire surface the allantoic vascular mesh appears complete and rich, though sparse at the tip ends; the area in the vicinity of the embryo being especially favoured by an abundance of capillaries (Heuser, 1927). In due course allantoic blood vessels invade the territory of the amnio-chorion which makes contact with the maternal blood supply.

Measurements of the volume of the amniotic and allantoic liquids in the pig are given by Paton, Watson and Kerr (1907) and Wislocki (1935a). The amniotic fluid increases steadily up to the third month (200 ml.). Thereafter the amount of fluid is subject to wide variations, but there is a tendency for it to decline somewhat in volume. The allantoic fluid, on the other hand, increases with amazing rapidity during the first third of pregnancy, rising to the level of 200 ml. or more before the embryo is over 30 days old, but like the amniotic fluid it declines towards the end of gestation and then does not often exceed 100 ml. Wislocki (1935) commenting on the mode of formation and ultimate fate of these fluids in the sow suggests that the unparalleled rise in the volume of the allantoic fluid to the level of 200 ml. or more before the embryo is over 3 cm. long cannot be reconciled with the theory of a mesonephric origin, but must, doubtless, come in whole or part from the well vascularised wall of the expanding allantois. Available evidence (Warwick, 1928, and personal observations) shows that the weight of the foetal membranes increases at a fairly uniform rate throughout the gestation period reaching a maximum of a little over 200 grms. at term.

The yolk-sac and the chorio-vitelline placenta

The yolk-sac is of considerable size and very vascular in young embryos. For a time it is fused ventrally with the chorion and relinquishes its connection only when the extra-embryonic coelom extends into the trilaminar omphalopleur and destroys the continuity between the yolk-sac and chorion. This close topographical relationship between the yolk-sac and chorion which is denied by Zietzschmann (1924) and others, constitutes, according to Heuser (1927), a yolk-sac placenta which may exercise both absorptive and secretory activities simultaneously. In early stages, the yolk-sac extends throughout the length of the chorion, but in the later part of the gestation period it is displaced by the developing allantois, shrinks to hardly more than a thin membrane, and finally disappears before the end of a gestation which lasts about three months, three weeks and three days. Nevertheless, evidence can be seen, even in late stages, for example when the embryo measures 10 cm. (about 55 days), of the atrophic, folded, central parts which may still have a diameter of 1 cm.

The chorio-allantoic placenta

The histology of the pig's placenta has been described by several investigators (Robinson, 1904; Assheton, 1906; Bonnet, 1907; Grosser, 1909 and 1927; Goldstein, 1926; Heuser, 1927; Brambel, 1933; Hutter, 1935; and Wislocki and Dempsey, 1946b). The present account of it is based on their observations supplemented by personal findings on a series of placentae ranging in age from eighteen days to near term (28 cm. foetus).

For purposes of description it will be convenient to consider the maternal and foetal portions in turn.

The maternal placenta. The maternal part of the placenta is composed of a typical mucous membrane thrown into numerous, very conspicuous, transverse folds or ridges. These folds are exceedingly vascular and attain a maximum development about the eightieth day of gestation. A comparison of Figs. 15, 19 *a* and 19 *b* will show the extent of the changes that occur during the course of pregnancy.

The endometrium may be divided into two fairly well defined layers (Corner, 1921*b*; Heuser, 1927):—a superficial zone with few gland tubules, and a basal zone next to the muscularis, densely packed with the fundic portions of many tubules. The glandular epithelium is ciliated throughout the neck region and is taller in the superficial tubules than elsewhere. The extent and manner of branching of the glands in the pregnant sow have been described in detail by Heuser (1927).

The uterine mucosa contains no special caruncular areas such as are found in the cow, sheep and goat, and is lined throughout with a simple one-layered non-ciliated epithelium. The subepithelial tissue is gelatinous, and early in pregnancy it begins to increase in thickness by a widening of the lymphatics and blood vessels and a new formation of capillaries. During pregnancy the glands are greatly hypertrophied and do not at any time show signs of degeneration. The cells are characterised by intense cytoplasmic basophilia, a large number of mitochondria (Wislocki and Key, 1921), much acid phosphatase, iron, calcium and lipoids but relatively little fat and no glycogen (Wislocki and Dempsey, 1946*b*). Corner (1921*b*) and Brambel (1933) report having also obtained negative results for stainable glycogen. The glands elaborate a copious serous secretion said to contain much stainable acid phosphatase

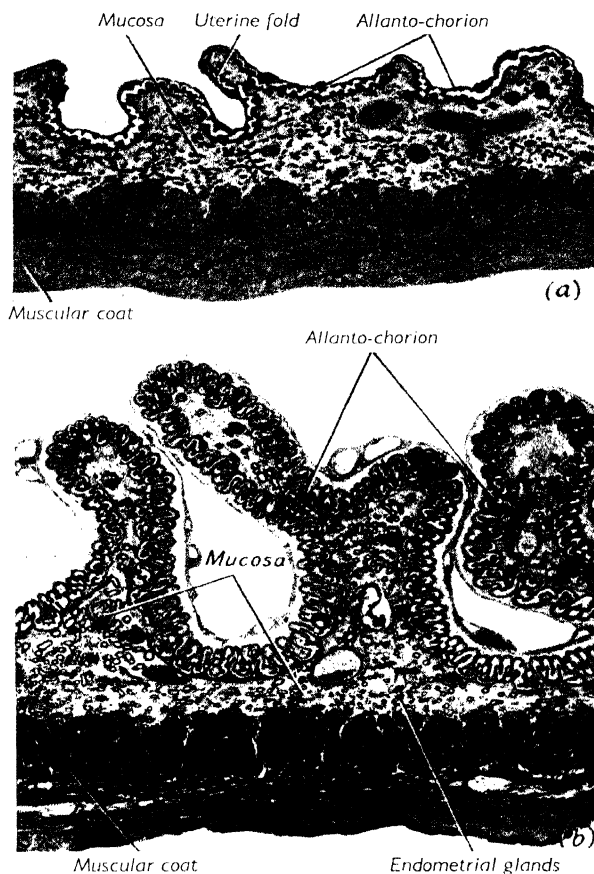


Fig. 15, 19.—Photomicrographs of sections through the allanto-chorion and uterine wall, showing increase in size and complexity of mucosal folds with ageing. (a) From a sow 22 days pregnant. (b) From a sow 50 days pregnant. x 12.

and iron. In the endometrium of the gravid uterus, Wislocki and Dempsey have also described the presence of alkaline phosphatase in the endothelium of the capillaries and venules as well as in the stroma. In the latter it is only occasionally present in the stromal cells. Wislocki, Deane and Dempsey (1946) suggest that the enzyme may have a predilection for the first cellular barrier intervening between the maternal and foetal circulations. However, this hypothesis may not be of universal application. (See Hard, 1946.)

As in the sheep, horse, whale, porpoise, peccary and some of the lemurs, the glandular orifices are covered by domes of trophoblastic cells—the *areolae*—which

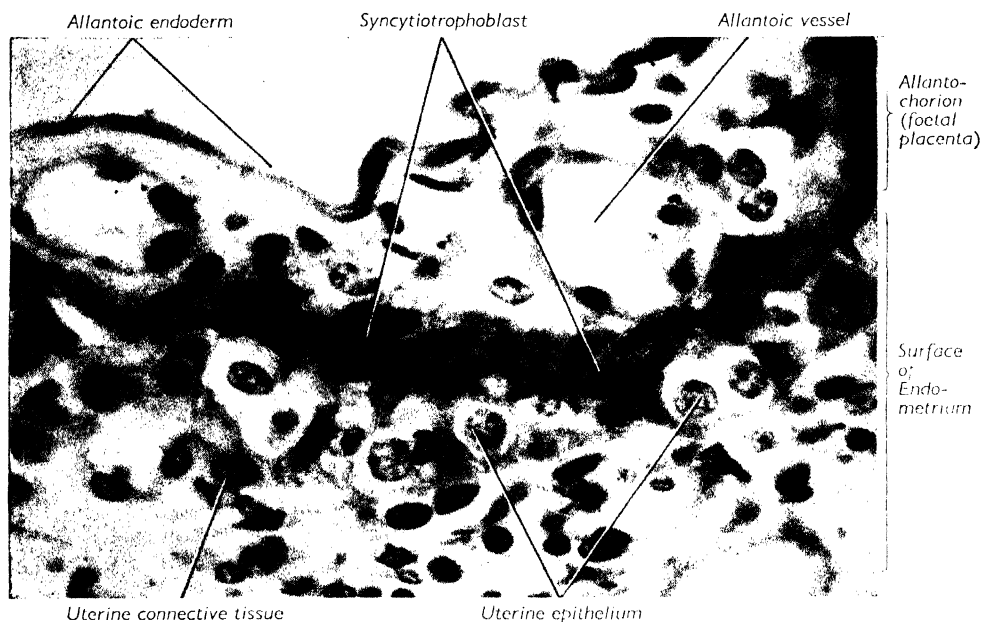


Fig. 15. 20—Photomicrograph of a section through the placenta of a sow 22 days pregnant, showing the syncytial trophoblast with trophoblastic processes between uterine epithelial cells. Uterine epithelium intact but degenerate. 550.

absorb the secretion (uterine milk) and transmit it as nutriment for the developing embryo by the allantoic vessels (see Fig. 15. 26). The constituents of the uterine milk are referred to later in this chapter.

It is generally agreed that the uterine surface epithelium remains intact, but as Assheton first showed there is good evidence that by the eighteenth day it is reduced to a thin layer (Fig. 15. 20). A week later, however, it again appears normal and is formed of low columnar cells to the ends of which the trophoblast fits closely sending protoplasmic processes between the cells. Our material confirms completely Robinson's (1904) contention that these processes may, in the early stages at least, reach past the epithelium into the underlying layer of dilated capillaries. The uterine epithelium remains quite uniform for a time, but begins to differentiate as the endometrial ridges commence to divide; over the summits of the mucosal septa the epithelium is columnar while in the depths of the grooves beneath the

growing tips of trophoblast the epithelium is cuboidal. At the same time a very close relationship develops between the uterine surface epithelium in the depths of the grooves and the underlying uterine capillaries. Here the uterine capillaries in many places displace the cuboidal epithelial cells and compress their cytoplasm into thinned out plates. These relations are illustrated in Fig. 15. 22 *b*. Measurements of the maternal epithelium (Gellhorn, Flexner and Pohl, 1941) show that

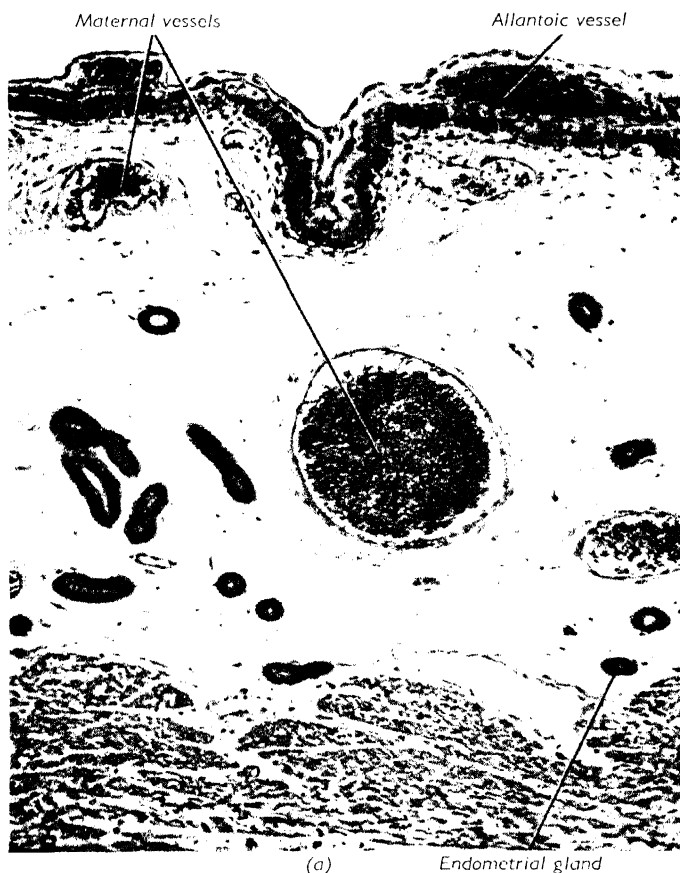


Fig. 15. 21-- (a) Section through allanto-chorion and adjacent part of endometrium from a sow 22 days pregnant. $\times 120$.

it changes from a height of $18\ \mu$ at mid-pregnancy, to $13\ \mu$ at 100 days, and $10\ \mu$ just before term and personal observations are in substantial agreement with theirs.

Foetal placenta. The allanto-chorion forms the foetal part of the placenta and even in its fully developed condition is a relatively simple structure histologically. For convenience it may be divided into two parts, areolar and interareolar areas. At first its surface is comparatively smooth (Fig. 15. 21 *a*), but subsequently it obtains a greater surface of contact by a series of concertina-like foldings (Assheton, 1906), which fit between the ridges of the uterine mucosa (Fig. 15. 21 *b*). These folds are

later subdivided into small delicate microscopic ridges which have the appearance of "villi" and have been conveniently but erroneously so designated (Fig. 15. 21 c). Typical villous structures are, however, occasionally present on the chorion (Heuser, 1927), but the number of these is considered too small to increase materially the absorptive surface. The primary and secondary ridges of the chorion

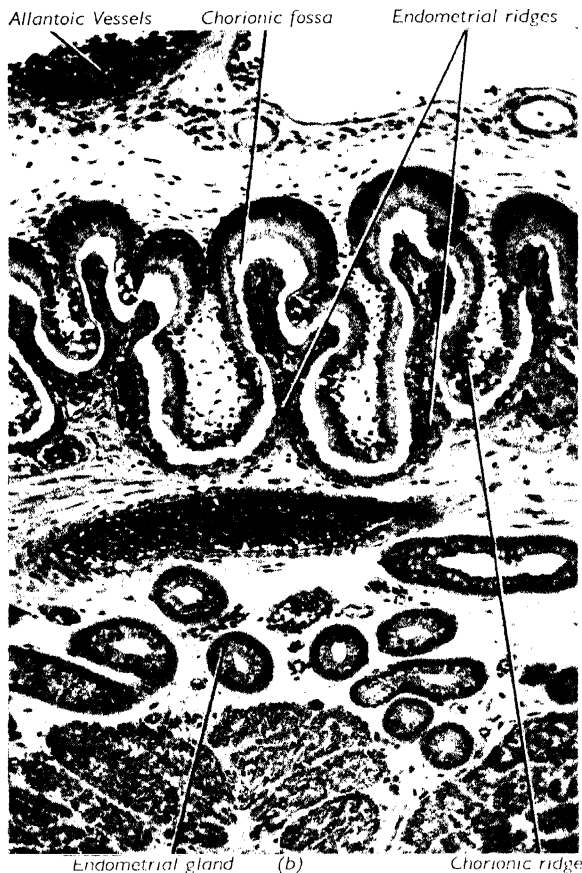


Fig. 15. 21—(b) Section through allanto-chorion and adjacent part of endometrium from a sow 75 days pregnant. 120.

are formed of trophoblastic ectoderm and contain allanto-chorionic mesenchyme. They are exceedingly vascular and fit into correspondingly shaped fossae in the uterine mucosa.

Regarding the manner of union of the chorion and mucosa, Wislocki and Dempsey (1946b), agree with Heuser (1927) that the primary and secondary folds along with the microscopic villi, matched by corresponding uterine depressions, are sufficient to anchor the layers together. They suggest, moreover, that during life the interlocking folds, which it will be recalled are very vascular, may possibly be distended with blood and tissue lymph, this swelling contributing materially to holding the two surfaces in close apposition.

A much debated question is whether there is always a space between the trophoblast and the epithelial cells lining the depressions of the uterine mucosa. Bonnet (1907) and Grosser (1909), both described the presence of a broad band of uterine milk lying between the chorion and the uterus and

largely through their influence this view is still held by some workers, although a contradictory account was given by Robinson as early as 1904. Indeed, Abramavich (1926) states that there is no actual fusion at any point between the maternal and foetal portions of the placenta; the two being separated by a thin layer of mucus approximately 80μ thick. The presence of a mucous secretion has been vigorously denied by both Corner (1921b) and Heuser (1927).

One of the difficulties in the investigation of the structural arrangement of the foetal membranes in the sow, is the fact that they are very readily separated from

their connections with the maternal tissues, so that it is not easy to preserve their relations in fixed material. However, from the histological point of view, it is difficult to accept the conclusion that in life they are not in the closest apposition, since it has been amply demonstrated by many competent embryologists that the cells are certainly provided with many interlocking processes, which indicate an original intimate relationship.

Furthermore, Figs. 15. 22 *a* and 22 *b* attest to the closest connection being maintained at all stages of pregnancy between foetal and maternal tissues and the apparently persisting uterine lumen between the two surfaces figured by Grosser (1909) and extensively reproduced by later writers must be regarded as artificial.

The trophoblast is single layered throughout and is supported by allanto-chorionic mesoderm in which are found the allantoic vessels. After the first three weeks it forms a syncytium (Fig. 15. 20). Later (28 days), however, it again appears normal and is formed of columnar, evenly granular trophoblastic cells (Fig. 15. 22 *a*). A further differentiation takes place at the time when the chorionic ridges begin to be subdivided; in the depths of the chorionic fossae between the chorionic ridges, the cells remain tall columnar and are provided with brush borders (Fig. 15. 23), whilst on the sides and over the summits of these ridges they become

cuboidal, flat or even syncytial (Fig. 15. 24), regaining their simple columnar form as term is approached (Fig. 15. 22 *b*). In the ripe placenta there are wedge-shaped processes from the cytotrophoblast which lie opposite the gaps between the protruding uterine epithelial cells and extend for some distance towards the basement membrane. These processes are similar to those in the syncytiotrophoblast of the early placenta (Fig. 15. 20), but whereas the latter are more prominent and reach almost as far as the subepithelial capillary layer of the endometrium, those of the cellular trophoblast extend for much shorter distances (*cf.* Figs. 15. 20 and 22 *b*).



Fig. 15. 21 (c) Section through allanto-chorion and adjacent part of endometrium from a sow near term. $\times 120$.

Another important question pertains to the very close relationship which develops during the last two-thirds of pregnancy, between the foetal and maternal capillary plexuses of the interareolar areas. At numerous places near the apices of the chorionic ridges, the foetal capillaries penetrate and run among the epithelial

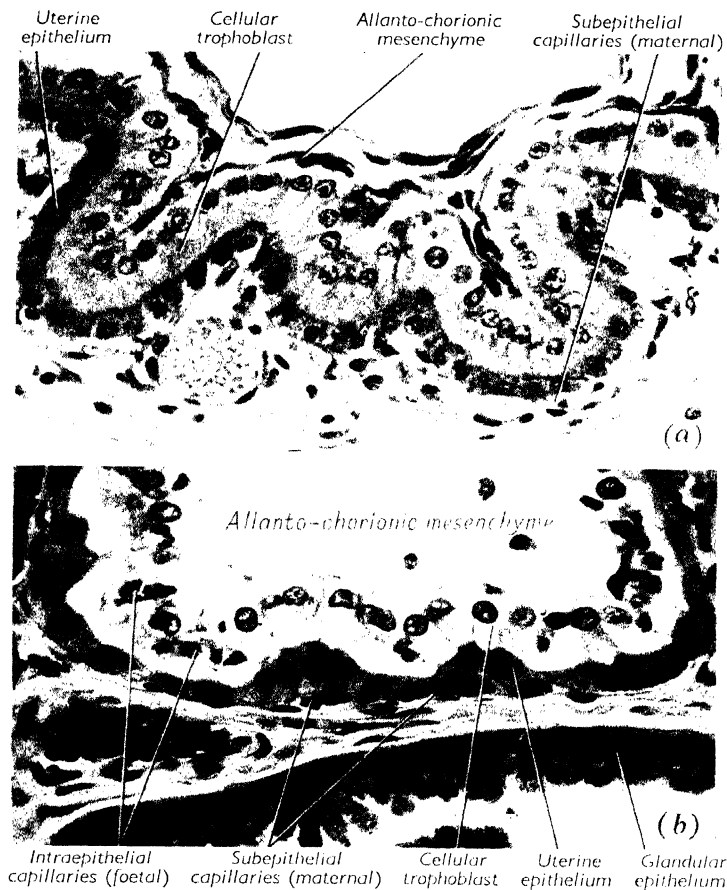


Fig. 15. 22 —Sections through allanto-chorion and adjacent part of endometrium, showing nature of foetal-maternal union. (a) From a sow 28 days pregnant. $\times 385$. (b) From an animal near term. $\times 400$.

cells (Fig. 15. 24). These changes begin about the end of the seventh week of pregnancy and gradually become more prominent during the remainder of the gestation period. Often the capillaries come to lie so near the surface that only the thinnest laminae of epithelial cytoplasm plus a delicate network of argyrophil fibres separate the blood vessels from the maternal tissues, where enlarged capillaries also lie against but without penetrating the uterine epithelium. In the chorionic fossae on the other hand, the individual columnar cells are never deformed by penetrating capillaries. Thus the interareolar area is divided into two parts, the intraepithelial

capillary nets, constituting the sites of transmission of the more diffusible substances (haemotrophe), and the relatively avascular columnar cells of the fossae which take up the less diffusible substances transmitted or secreted by the uterine epithelium (histotrophe).

Of further interest in this connection are the observations of Goldstein (1926) who re-investigated the blood supply of the chorion. He expresses the opinion that the invasion of the trophoblast by the capillary plexuses is often so complete as to lay bare the endothelium, an arrangement which he designates "epithelio-endothelial" in accordance with Grosser's terminology. However, a study of his published photographs as well as the examination of our own material do not bear out this assumption. His results have also been criticised by Grosser (1927), Heuser (1927) and Töndury (1944), and more recently



Fig. 15. 24—Chorionic folds from a sow 60 days pregnant, showing intra-epithelial capillaries distended with foetal blood corpuscles. $\times 335$.

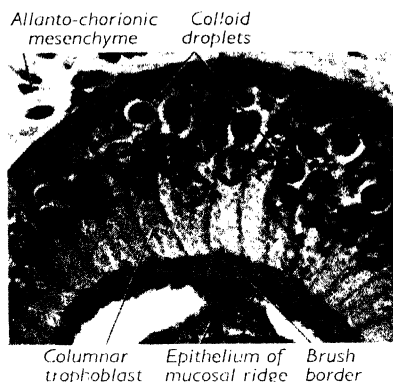


Fig. 15. 23—Columnar cells of chorionic fossa, showing typical colloid droplets and brush border. $\times 560$.

by Wislocki and Dempsey (1946b) who state that "each vessel is enclosed in a delicate network of argyrophil fibres which delimits the vessel wall from the surrounding epithelial cells and make it quite apparent that there is invariably a thin lamina of epithelial cytoplasm between the wall of the vessel and the outside." In Fig. 15. 24, this relationship is illustrated in the placenta of a sow 60 days pregnant. It must be admitted, however, that where the epithelium is tunnelled with capillaries, cell outlines are often lost and the summits of the chorionic ridges are then characterised by what appears to be a vascular plasmoditrophoblast not unlike that described in the placenta of the peccary by Wislocki (1931). This invasion of the trophoblast with capillaries was first described by Tafani (1886) in the pig, and independently again

by Goldstein in 1926. It is not peculiar to the placenta of the pig. Nor can it be said to have developed in special association with the epitheliochorial

placenta, for, it appears to be a structural arrangement commonly encountered in the placenta of ungulates (syndesmochorial), carnivores (endotheliochorial), rodents (labyrinthine haemochorial) and man (villous haemochorial). See also pages 134 and 278.

The observations of Gellhorn, Flexner and Pohl (1941) and Flexner and Gellhorn (1942) on the transfer rate of radioactive sodium across the placenta of the sow agree with the morphological developments just described, inasmuch as there is a steady increase in the transfer rate across the placenta during the last two-thirds of pregnancy with no sharp drop as term is approached. These and other data of Flexner and his associates suggest that there is a correlation, (1) between changes in placental structure and changes in the rate of transfer of sodium at different periods of gestation, (2) between transfer rate of sodium and the morphologic type of placenta, and, (3) between the relative growth rate of the foetus and the rate of transfer of sodium to the foetus. It is not within the province of this chapter to discuss the transmissibility of substances to the foetus, although the data here cited have a direct bearing upon the question. This aspect of the problem is treated in Chapter 16.

Histochemical reactions of the chorion.—Wislocki and Dempsey (1946b) report differences in the histochemistry of the cells of the chorionic ridges and the columnar cells of the chorionic fossae. They have shown that the cuboidal cells of the ridges exhibit pronounced basophilia during the first half of pregnancy, a moderate amount of iron, increasing amounts of calcium and traces of glycogen in early pregnancy. The cells exhibit no stainable fat and no acid or alkaline phosphatase. Brambel (1933) also failed to find fat and glycogen. In the columnar epithelium of the fossae Wislocki and Dempsey have described the localization, within the distal half of the cells, of glycogen, calcium, iron and alkaline phosphatase (confined to the brush border), whereas fat as well as three cytoplasmic elements with an affinity for basic dyes (ribonucleoprotein, colloid droplets and crystals), are said to be confined to the basal half of the cells (Fig. 15. 23). Moreover, they have confirmed the findings of Höpping (1937) that a temporary storage of calcium associated with glycogen and alkaline phosphatase occurs in the stroma of the allanto-chorion during a good part of pregnancy. This deposition of calcium they considered to be unrelated to the petrifications observed at term in the allanto-chorion of the sow by Brambel (1933) and Hutter (1935). The histochemical reactions of the chorion and endometrium as observed in the course of the present study are, with minor exceptions, confirmatory of the findings of Wislocki and Dempsey (1946b).

Allanto-chorionic differentiations.—The most conspicuous structural differentiations on the surface of the pig's chorion are the areolae. Embryologists have long been aware of the fact that these structures which arise as minute circular areas opposite the mouths of the uterine glands, represent regions of the chorion which may have become specialised in connection with the absorption of the secretions elaborated by the glands and the uterine epithelium (von Baer, 1828 ; Turner, 1875 ; Grosser, 1927). Together with the uterine depressions which fit over them, they form definite organic units isolated structurally from the rest of the placenta. Two morphological types of areolae (called "rosettes" by Abromavich, 1926) are differentiated on the chorion of the 10 to 12 mm. embryo, the *regular areolae*, the ones usually described in the literature, which are circular,

opaque, and complexly folded, cup-like invaginations of the chorionic surface (Fig. 15. 25 *b*), and the *irregular areolae* (called pits by Heuser, 1927), translucent plaque-like structures with little tendency to invagination, and as much as fifteen times the diameter of the regular areolae (Fig. 15. 25 *a*). During very early stages of gestation, the surface of the regular areolae is flat (Fig. 15. 26 *a*), but as gestation advances the flat surface is invaginated and becomes complicated by ridges and papilla-like structures (Fig. 15. 26 *b*). The two structural types, occurring in large numbers (8000-9000), are distributed over the entire chorion and are relatively more numerous in mid-pregnancy (approximately 6 per square centimetre) than at term (approximately 2.5 per square centimetre).

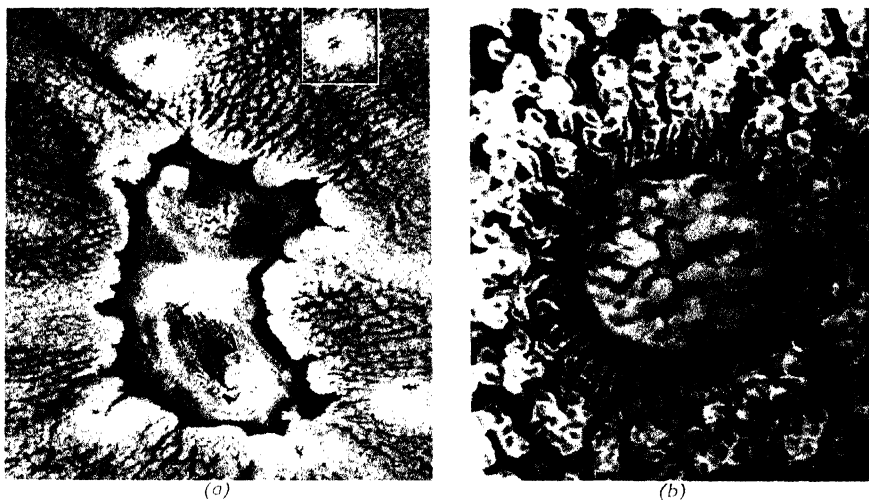


Fig. 15. 25:— (a) Uterine surface of piece of chorion (Embryo, 160 mm.), showing chorionic villi, one irregular areola and 7 regular areolae. Area enclosed in rectangle is shown at higher magnification in Fig. 15. 25 (b). $\times 2\frac{1}{2}$. (b) Detail of area enclosed in Fig. 15. 25 (a), showing regular areola and radial arrangement of chorionic villi. $\times 15$.

Microscopically, the areolae have a different type of epithelium and vascular pattern from the interareolar area, details of which are described by Abromavich, (1926); Goldstein, (1926); Heuser (1927); Brambel (1933); and Wislocki and Dempsey (1946b). The epithelial cells are mostly simple columnar. All have small darkly staining nuclei and a pale cytoplasm containing numerous vacuoles; the contents of which stain like the secretion (uterine milk) located in the areolar cups. The cells are further characterised by long lasting deposits of glycogen and dense concentrations of iron and acid phosphatase (Wislocki and Dempsey, 1946b). The blood supplied to the folds in the wall of the cup comes from a capillary ring, linked with the capillaries in the interareolar area, and is drained by a number of venules depending upon the number of folds making up the wall of the cup. In the areolae, the capillaries never displace the epithelial cells as they do in the chorionic ridges of the interareolar areas.

Similar structures to those under discussion are described for the intercotyledonary area of the cow, sheep, goat and giraffe, the paraplacental region of the bitch and cat (Fig. 15. 57 *b*) and on the diffuse placenta of the mare, tapir, peccary,

porpoise, sei whale and the pangolin. Likewise the chorionic vesicles of the lemurs (Hubrecht, 1894; Strahl, 1899; Gerard, 1932; Hill, 1932), the American mole (Mossman, 1939a) and of the cat (Fig. 15. 57 *a*) constitute an apparatus obviously similar in its general features to the areolae of the pig and ungulates generally.

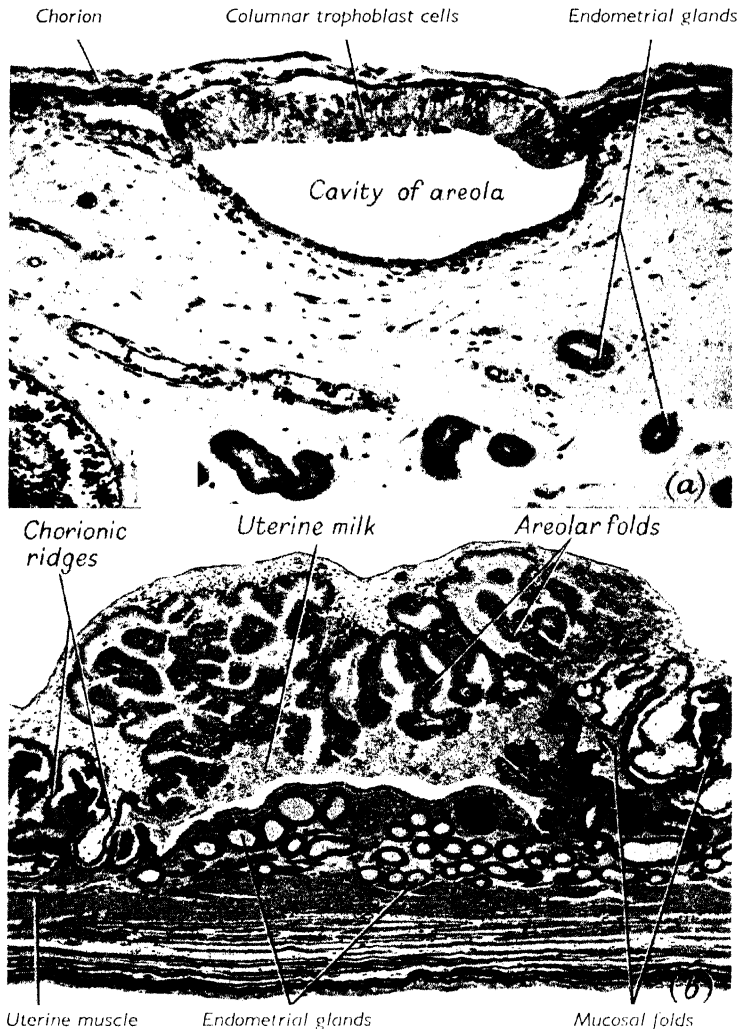


Fig. 15. 26—Sections of two regular areolae. (a) From a sow 23 days pregnant. $\times 130$. (b) From an animal at term. $\times 43$. Note absence of uterine papillae, unlike irregular areolae where also the epithelium produces a mucoid secretion and forms symplasma.

Other structures varying from slightly invaginated pockets to closed off cysts, or bodies suspended by epithelial cords, or entirely free have been described on the interareolar areas (Heuser, 1927; Brambel, 1933). These structures are less numerous, appear later, and are more varied in their distribution than the areolae

(Brambel). From their resemblance to the initial stages in the formation of the yolk-sac hippomanes in some lemurs (Hamlett, 1935a), Wislocki and Dempsey (1946b) state that they may represent the intermediate stages in the formation of "Hippomanes" in the sow. Nevertheless Brambel's observations on the distribution of the cysts do not support this assumption, inasmuch as the "hippomanes" are restricted to the ends of the chorionic sac—areas which are, as a rule, free of cystic formations.

The Foetal Membranes and Placenta of the Horse.

The structure and development of the foetal membranes and placenta of the horse at consecutive stages of pregnancy still await an exhaustive examination. Most of the available data will be found in the papers of Turner (1875), Bonnet (1889a; 1907), Ewart (1898; 1915), Kolster (1903), Assheton (1906), Thieke (1911), Schauder (1912), Zietzschmann (1924), Catchpole and Lyons (1934), Cole and Goss (1943) and Krölling (1940), and the evidence which is presented in this section is, in the main, a confirmation of their observations.

The placenta of the mare is distinctly a diffuse epitheliochorial one and like that of the sow consists of two epithelial layers which are closely apposed at certain points and variously separated by irregular spaces at others. It further resembles that of the pig in its non-deciduate behaviour at birth. Placentation in the mare differs, however, fundamentally from that of the pig (1) in that attachment is completed relatively late, (2) in the absence of the extensive amnio-chorion which characterises the placentation of the pig, (3) in the persistence, until relatively late stages, of a well-developed chorio-vitelline placenta as contrasted with its insignificant rôle in the pig and, (4) in the slightly more complex structure of the placental labyrinth.

The placentation of the mare bears a certain resemblance to that of some of the ruminants, in that the villous epitheliochorial condition is supplemented by syndesmochorial regions in the tissues comprising the "endometrial cups" (*see* below). These latter are temporary structures only, and are gone by the end of the fifth month of pregnancy, the duration of which for different breeds, ranges from about 329 to 345 days. However, in the curious development of the endometrial cups, combined with the changes in external form which they undergo as pregnancy advances and in their probable implication as sites of production of serum gonadotrophin (P.M.S.), the placenta of the mare differs markedly from any type so far described. There is here a wide field for future work and it would be interesting to know to what extent these conditions apply to the placenta of the donkey and the zebra (*see*, however, Asdell, 1946).

Implantation and the yolk-sac placenta.

In reference to the time of attachment, the blastocyst in the mare lies relatively unattached in the uterus until a comparatively late stage of development. Ewart (1915) places the end of the seventh week as the time when chorionic villi begin to penetrate into the uterine sulci. At this stage the attachment is most frail, the membranes being held in position mainly by the pressure of the foetal fluids. Further evidence of the belated attachment of the blastocyst in this species is furnished by Kolster (1902) and Catchpole and Lyons (1934), who find that even

after this period the whole chorionic sac will fall away from the uterus under its own weight. Analysis of the records of 10 mares killed between the eighth and ninth weeks of pregnancy is in harmony with these observations. However, at about the tenth week this operation is almost impossible without rupturing the membranes, for the chorionic villi will have penetrated further into the mucosal folds, resulting in a closer attachment. Zietzschmann (1924) states that by the fourteenth week (embryo length 15.5 cm.) complete attachment of the chorionic sac to the uterine mucosa is attained.

To meet the requirements of this early growth period, when the blastocyst lies relatively free in the uterine cavity, there is a copious production of "uterine milk" (*see also* p. 209). It is derived from the break-down of maternal tissues supplemented by the secretions of the uterine glands and of the intact portions of the uterine mucous membrane. This secretion accumulates in the space between the trophoblast and the endometrium, and recent observations (Cole and Goss, 1943; Rowlands, 1947; and the author) have shown that between the seventh and fourteenth weeks of pregnancy, it possesses, in addition, an unusually high concentration of gonad stimulating hormone. The elaboration of large amounts of "uterine milk" lasts until the anchorage of the chorionic villi in the uterine mucosa has become so intimate that the uterine lumen is practically obliterated (fourteenth week) and the foetus is able to take advantage of nutrient materials circulating in the maternal blood. Thereafter as the need for histotrophe diminishes, the abraded uterine surface is restored. The destruction of the uterine epithelium is confined to the peculiar oval areas of the mucosa—the so-called "endometrial cups."

In the early stages of development, the splitting of the mesoderm is delayed and never complete; hence the yolk-sac never develops an independent wall of its own as in the pig and sheep. Moreover, the mesoderm does not completely invade the lower polar regions of the blastocyst until relatively late in pregnancy, so that, for a time, both a bilaminar omphalopleur and a chorio-vitelline placenta are present and the blastodermic vesicle is attached to the uterine mucosa by the trophoblast covering the lower pole of the ovum. This yolk-sac connection is of a very slender kind, and is the only one which exists up to the fifth week. The trophoblastic wall of the vesicle at the end of the third week differs from that of the sheep and pig and other Ungulates. Up to the sinus terminalis it consists of typical columnar cells; beyond the sinus there are (1) groups of tall columnar cells arranged to form discs which probably help to fix the blastocyst to the lining of the uterus; (2) groups of columnar cells with sac-like processes placed around the discs and especially in shallow depressions beyond the sinus terminalis which probably lie opposite the openings of the uterine glands and are concerned in taking up the more solid particles of uterine milk (Ewart, 1915).

At the fifth week additional means of securing the embryo to the mucosa are evident by an increase in the extent of the chorio-vitelline attachment. There is also a thickening of the trophoblast about 7 mm. wide, placed around the equator of the chorionic sac. This trophoblastic annulus adheres to the uterine wall, and so strengthens the original anchorage. About the end of the sixth week the attachment of embryo to uterus is again becoming frail, for the yolk-sac placental area has become less, while the annulus has shifted from the equator to near the pole. It is at this stage that the change from the chorio-vitelline to the more

advanced chorio-allantoic placental circulation is being effected, and it is usually regarded as a critical period in development, when the foal is more liable to "slip" than at other times. The cause of this "slipping," especially in the case of twins, may not of course be unrelated to the appearance in the blood serum of the dam of unusually high concentrations of gonad stimulating hormone.

The yolk-sac in the horse, apart from playing an important rôle in the nutrition of the embryo, is concerned to a variable extent in blood formation. Consequent on the increase in size of the embryo and the enlargement of the exo-coelomic cavity, the chorio-vitelline area is gradually devascularised and the yolk-sac splanchnopleur increases markedly in extent. From it, as well as from the adjacent trilaminar omphalopleur, numerous, club-shaped villi are given off projecting into the yolk-sac cavity. The endodermal wall of these villi are composed of large cells, the cytoplasm of which becomes filled with small eosinophilic granules. The vitelline vessels enclosed in the villi contain numerous primitive blood cells and megakaryocytes and evidently constitute a highly active haemopoietic centre. Moreover, the columnar absorptive, trophoblastic cells, opposite and beyond the sinus terminalis engulf maternal corpuscles and other cell material, and transmit their products of resorption through the mesoderm and the thin endodermal layer into the yolk-sac cavity, where they are taken up by the enlarged endoderm-cells of the villi of the yolk-sac. It is interesting to note in this connection, that from the sinus terminalis, outward over the somatopleur, the mesoderm has the form of a thin structureless membrane suggestive of the membrane of Reichert found in the Rodentia. This membrane may be of considerable importance as an element of the yolk sac placenta; it should be more completely studied.

The amnion, allantois and umbilical cord.

The amnion appears to be completed relatively later in the horse than in the pig and other Ungulates, and is, in fact, only just completed at the end of the third week of pregnancy. Up to the eighth week it is closely applied to the embryo and the volume of amniotic fluid does not exceed 2 to 5 ml. Although the volume of amniotic fluid increases during the ninth and tenth week, the rate is comparatively slow (*see* Table IV). However, this is followed by a period of more active

TABLE IV

Age (days)	Amniotic fluid (ml.)	Allantoic fluid (ml.)
46	1	160
61	10	1405
62	18	1050
63	19	1270
68	15	1900
71	8	2130
72	9	2050
74	27	2050

growth, and the sac becomes relatively wide, so that by the middle of pregnancy its contents about equals that of the allantois, while later on the allantoic fluid again predominates. At term, the amount of amniotic fluid varies from 3 to 5 litres.

From the tenth week onwards, the inner surface of the amnion begins to be studded with numerous, rough protuberances—the “amniotic pustules”; their sizes show considerable variation and they are formed by local heapings-up of the amniotic ectoderm in which large quantities of glycogen are stored. Their function remains unknown.

Like the amnion the development of the allantois is retarded; at the twenty-first day it is a minute endodermal diverticulum, but by the twenty-eighth day

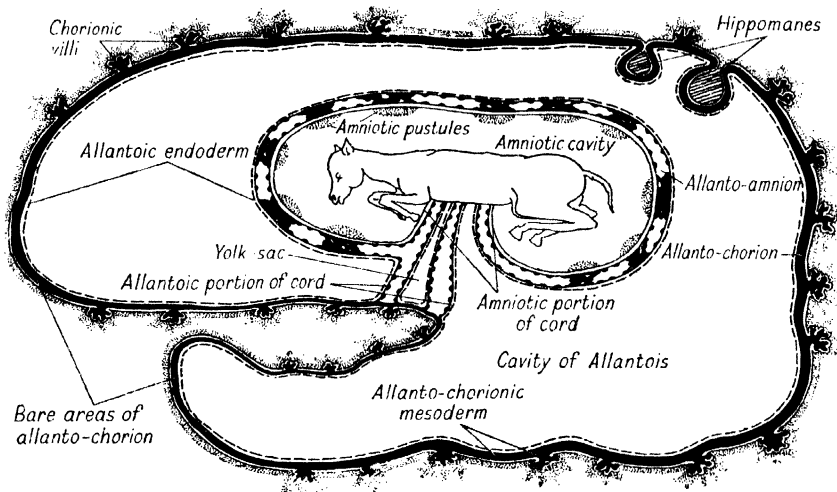


Fig. 15. 27 —Diagram of allanto-chorionic sac of the horse towards the middle of gestation. Note that the Hippomanes have been misplaced in the drawing and should appear in the neighbourhood of the Yolk-sac.

it has completely surrounded the amnion and expanding now in the direction of the ab-embryonic hemisphere as far as the sinus terminalis, practically encloses the yolk-sac in such a way that the latter is almost, but not completely, walled off from the chorion by the two walls of the allantois and its contained liquor. The allantois is completely formed by the sixty-third day and has fused over its external surface with the chorion, thereby forming an extensive allanto-chorion. In the zone of contact with the amnion the inner wall of the allantois fuses with the amnion to form allanto-amnion which is accordingly vascularised. The volume of allantoic fluids varies widely in health and with the breed of animal. Zietzschmann (1924) gave a range of 8–15 litres for the allantoic liquor at term.

The anatomical peculiarities of the allanto-chorion in the horse (Fig. 15. 27) combined with the enlargement of the amnion and the extension of the amniotic sheath over the umbilical cord are responsible for the subdivision of the cord into allantoic and amniotic portions. The former extends from the allanto-chorion to the amnion, and the latter through the amniotic cavity to the umbilicus. The length of the umbilical cord of the horse varies with the breed, being generally

about equally divided between amniotic and allantoic portions. Zietzschmann (1924) gave the absolute length as about 100 cm., while Williams (1940) found an average of just under 50 cm. for a series of thirty thoroughbred mares. Amniotic pustules are abundantly present on the surface of the cord and the latter exhibits a narrowing just before it reaches the edge of the skin of the foetus (Hauptmann, 1911). A sphincter muscle is present. For a further discussion of this and related problems the review of Franklin in the book on the Foetal Circulation by Barclay, Franklin and Prichard (1944) should be consulted.

The histology of the chorio-allantoic placenta.

During the early stages of development of the placenta, the chorionic surface is relatively smooth, but folded in accommodation to sulci of the uterine mucosa.

Primary villi Opening of uterine gland Uterine milk in uterine lumen Areolae



Fig. 15. 28—Section through the allanto-chorion and endometrium of a mare 9 months pregnant.
48.

Between the seventh and eighth weeks of pregnancy, short simple villi appear over the middle segment of the chorionic sac, and the site at which these early structures are localised is marked by an hyperaemic reaction on the part of the uterus that can be recognised macroscopically. In the fully developed placenta, villi are present over the whole surface of the allanto-chorion except at each pole where the chorion is in relation to the openings of the Fallopian tubes, opposite the os uteri internum and also opposite the endometrial cups. The presence of bare areas at the poles of the chorion led Turner (1875) to point out the similarity of this condition, which Zietzschmann (1924) designates "placenta diffusa incompleta," to the type of placenta referred to as "zonary." Mossman (1937)

believes that the villous-free areas at the tips of the chorion, as well as at its vaginal extremity, are due to the absence of the stimulus necessary to induce villus formation, resulting from the failure of the chorion and mucosa to make direct contact at these points. Wislocki (1933a), on the other hand, believes that the membranous areas are associated with relatively avascular zones of the chorion.

Where villi are present in the ripe placenta they are considerably longer than in the earlier stages and in surface views are seen to be arranged around minute smooth, circular areas, the "areolae," which correspond to the openings of the uterine glands. Each villus consists of a main stem and many secondary branches

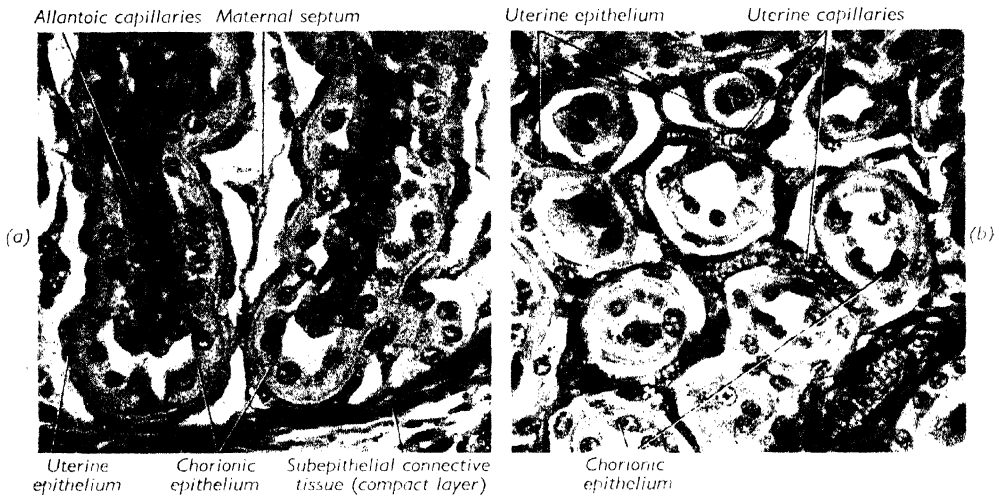


Fig. 15. 29 -- Photomicrographs of portions of villi attached to the surface epithelium of the uterus : from the same mare as in Fig. 15. 28. Notice the maternal and foetal capillaries. $\times 350$.
 (a) Longitudinal section. (b) Transverse section.

which interlock with correspondingly shaped pockets of the mucosa forming characteristic morphological units known as *cotyledons* (Bonnet, 1907). These cotyledons are separated by smooth interspaces. Fig. 15. 28 taken from the region of intimate interdigitation of endometrium and allanto-chorion, shows the broad zone of interpenetration of the spongy endometrium and the villous chorion. As will be seen from Fig. 15. 29 a the villi themselves are extremely vascular, a thin layer of dense connective tissue separates the placental labyrinth from the deeper lying, glandular zone of the mucosa. The lining of the maternal crypt in the zone of interlocking consists of a much flattened cellular layer of uterine epithelium closely applied to the cellular epithelial investment of the tips of the villi. The scanty supporting tissue of the walls of the crypt contain a rich plexus of capillaries (Fig. 15. 29 b).

The trophoblast cells at the base of the primary villi are decidedly taller than those at the tip ends which are cuboidal ; the former are provided with brush borders and in them are found minute fat droplets, pigment and large heavily

staining inclusions. Assheton (1906) described a similar arrangement of cells on the chorion of the sheep, and called these groups "arcades." It has been suggested that the intervillous area functions as a region of absorption of nutritive substances from the mother through the agency of the uterine glands (histotrophe) whilst the vascular villi constitute the sites of transmission of the more diffusible substances (haemotrophe).

Endometrial cups.

Distributed over a part of the endometrium in apposition to the chorion, specialised cup-shaped structures are found which have been referred to as "mucosal craters" (Schauder) or "endometrial cups" (Cole and Goss). As

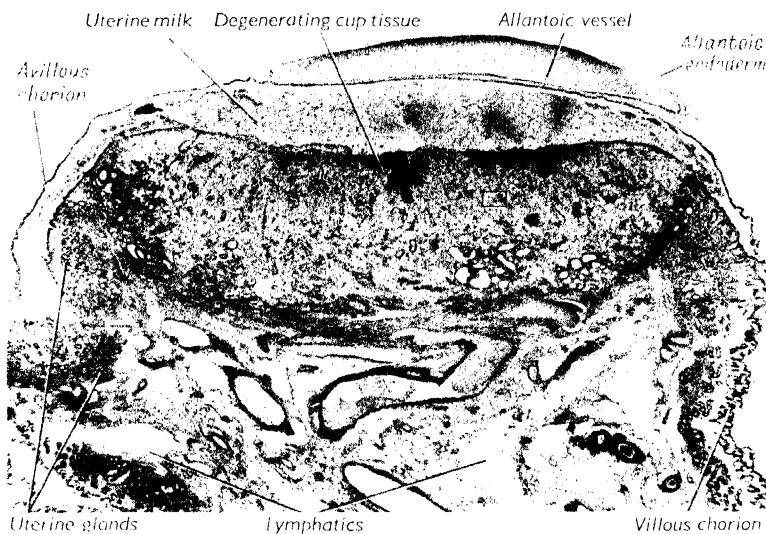


Fig. 15. 30. Section of an endometrial cup from a mare 63 days pregnant. $\times 82$.
Area enclosed in rectangle is shown at higher magnification in Fig. 15. 31.

early as 1912, Schauder reported their occurrence in pregnant uteri of the mare from the sixth to the twentieth week and associated their presence with the formation of uterine milk which, in his opinion, is derived in part from an early and extensive sloughing off of the superficial portions of the cup. These early observations have been confirmed from an examination of a good deal of material, and there is evidence, too, that the cups as well as the secretions they elaborate, contain high concentrations of gonad stimulating hormone (see also Cole and Goss, 1943 ; Rowlands, 1947).

The structures in question are limited to the fertile horn and vary in maximum diameter from a few millimetres to about 5 cm. (Fig. 15. 30). They occur most frequently at the junction of the body and horn of the uterus and most commonly occupy a circular or horse-shoe shaped area of the endometrium in the neighbourhood of the attachment of the yolk-sac. The allanto-chorion opposite the craters on the uterine wall is devoid of villi and the endometrium at these points also lacks

crypts. There is no attachment of the membranes and the uterine epithelium is wanting ; an irregular space, representing all that is left of the uterine lumen at the level of the cup, separates the trophoblast from the endometrium in the small area above the uterine depression. This space contains a granular, eosinophilic coagulum, presumably of glandular and connective tissue origin. Embedded in it are great numbers of degenerate uterine epithelial cells, erythrocytes and a moderate number of polymorpho-nuclear leucocytes. The ducts of the glands which open in the vicinity of the space secrete actively. The only thing about this early relationship which is not typical of a syndesmochorial placenta is the absence of any genuine connection between the foetal and maternal tissues.

As seen in the usual preparations stained by Haematoxylin and Eosin, the cups seem to be composed of three types of cells ; (1) numerous small round cells,

(2) large polyhedral cells lying between the ducts of the uterine glands, and (3) a few multi-nucleate giant cells which appear to be degenerating. The interglandular cells (Fig. 15. 31) are the conspicuous features of the cups when these are first formed and in their development they resemble most closely the decidual cells of the endometrium which are seen during pregnancy in other forms ; they differ, however, from "typical" decidual cells in that no stainable glycogen could be demonstrated, either by Best's Carmine method or by Bauer's method. They have rounded

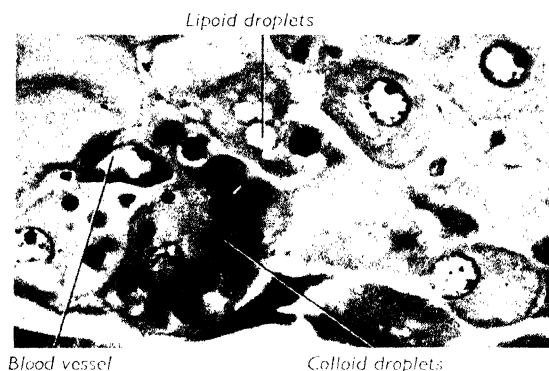


Fig. 15. 31—Detail of area enclosed in rectangle in Fig. 15. 30, showing polyhedral, interglandular cells filled with colloid and lipid droplets. $\times 540$.

contours, a granular, pale staining, vacuolated cytoplasm and pale, vesicular nuclei with prominent nucleoli. Transitions between these and typical plasma cells are common ; sometimes binucleate forms are encountered, but mitoses are rare.

Histochemically they exhibit a fine basophilic stippling, the ribose nucleoprotein occurring in the superficial portions of the cells ; whereas the central part of each cell is acidophilic, finely granular and often contains small amounts of greenish or brownish pigment. The cells are, as we have already observed, negative for glycogen but contain a moderate number of scattered, discrete particles of lipoidal material when stained with Sudan black. After regression has begun the cells show an increasing infiltration with neutral fat in addition to other lipoids—the fat droplets being especially large and numerous in the degenerating surface cells and in the detritic masses filling the cups. In addition to these features some of the cells possess characteristic cytoplasmic vacuoles which contain colloid droplets (Fig. 15. 31). Alkaline phosphatase is abundantly present but is restricted to the margins of the cells. The lymphatic system of the cups, as well as that of the mucosa, is enormously developed ; the blood supply is ample but details of distribution are lacking.

Hippomanes.

The name is given to small dark brown masses which are lodged in the foetal membranes or float free in the allantoic or amniotic fluids of the horse, cow, sheep and pig. In the horse, these curious bodies of the consistency of gluten—and elastic like it—are flattened, thinner at the borders than towards the centre, oval or irregularly circular and about 5 to 10 cm. in maximum diameter. They are sometimes found in the mouth of the foal; their function, if any, is still obscure.

The sectioned surface of a "hippomane" shows it to be divided into a dark, soft, outer, lamellated cortex and an inner medullary part composed of granular material, full of cell debris, globules of fat, and degenerating corpuscles. Thieke (1911) described their development in the horse and from his account one gets the impression that they are very generally and commonly known. According to Schauder (1912), the hippomanes may arise from the endometrial cups. He assumes that they become separated from the mucosa, and are shed into the uterine lumen whence they become encapsulated by chorionic folds to give rise to the pedunculated forms described by Thieke. These features are illustrated in Fig. 15. 32. For a description of the development of hippomanes in the cow and sheep, see Jenkinson (1913).

Placentation in the Lemuroidea.

A number of observations have been made in recent years on the foetal membranes and placentation of the *Lemuroidea*. Those of Hill and Burne (1922; *Chiromys*), Hill, Ince and Subba Rau (1928; *Loris*), Hill (1932; *Loris*, *Nycticebus*) and Gerard (1929, 1932; *Galago*) are the most comprehensive. It is indicated by these workers that many of the lemurs, including both the Oriental and African Lorisidae, have a simple diffuse, deciduate placenta, as Milne Edwards (1875) and Turner (1876b, 1878) first pointed out. However, Gerard (1932) has stated that in the development of its placenta *Galago demidoffi* exhibits certain unusual and quite unexpected features which distinguish it from all other Lemurs so far investigated. In this animal he described an area of endotheliochorial placentation surrounded on all sides by a diffuse epitheliochorial condition.

According to Jenkinson (1916)—who investigated the placenta in a species of *Lepilemur*—and Hill (1932), the relation between foetal and maternal circulations in the Lemuroid placenta is brought about by the production of vascular trophoblastic villi which fit into crypts lined by a persistent uterine epithelium. The placenta, therefore, resembles that of such an Ungulate as the pig; it is of the epitheliochorial type. Accordingly, the lemurs differ radically from all other Primates in which the gross shape of the placenta is discoidal or double discoidal, the endometrial relation deciduate and the type haemochorial. The following



15. 32. Pedunculated hippomane of mare. From allanto-chorion of 90 cm. foetus. Two-thirds natural size.

brief, general account of the placenta of the lemurs is essentially derived from Hill and Burne (1922) and Hill (1932): our own observations being confined to the examination of sections of the placenta of *Loris* and *Galago* (Figs. 15. 33 *a* and *b*), and a single pregnant uterus of Bosman's potto (*Perodicticus potto*).

As is evident, implantation in the Lemurs is of the central type with the foetal membranes remaining external to the uterine tissues. In *Galago demidoffi*, however, Gerard has described a temporary capsularis around the early blastocyst, formed presumably as a result of secondary enclosure and not of true interstitial implantation. In the Lemurs the chorionic sac is large and occupies both horns of the uterus, its surface, except over certain small restricted areas, being studded with villous processes which interlock with corresponding depressions of the vascular endometrium. The uterine epithelium persists throughout pregnancy and is said to be actively secretory in *Propithecus* (Turner, 1876b) and *Galago* (Strahl, 1899) but not so in *Chiromys* (Hill and Burne). In *Chiromys*, the basal ends of the uterine epithelial cells are directly applied to the walls of the subjacent capillaries and the same is also true of *Loris* and *Galago agisymbanus* (Strahl), but in neither of these is the subepithelial capillary plexus anything like so well developed as in *Chiromys*, although the corium of the mucosa is richly supplied with vessels. In *Galago demidoffi*, Gerard has described a "zone d'implantation" where the uterine epithelium has disappeared and is replaced by a layer of trophoblast cells. Here the maternal capillaries form a rich plexus which is actually situated between the ends of the foetal cells (Placenta endothelio-chorialis).

The uterine glands also remain functionally active. In *Propithecus* and *Galago* they open for the most part in groups, their openings in *Galago* being on depressed areas of the mucosa, opposite which there are present specially modified absorptive areas of the chorion (chorionic vesicles, Strahl, 1899, 1905b). In *Nycticebus* (Hubrecht, 1894) *Loris* (Hill, 1932), and probably also in *Chiromys* (Hill and Burne), on the other hand, their openings are more evenly distributed at the bottom of uterine crypts where the overlying chorion is again variously modified to form recesses (Hubrecht) or vesicles (Hill). The chorionic vesicles of *Nycticebus*, *Galago*, and *Loris* described by Hubrecht, Strahl, and Hill, are more highly differentiated than those of *Chiromys*, and Hill and Burne state that the vesicles in the latter may serve as reservoirs for the surplus glandular secretion rather than as absorptive organs. It seems, however, to have been conclusively proven that in some Lemurs, at any rate, they may represent intermediate stages in the formation of yolk-sac hippomanes (Hamlett, 1935a).

The chorionic villi are seen in their simplest condition in the *Lorisidae* where they appear as simple nodular processes separated by the extremely thin walls of the uterine crypts (Fig. 15. 33 *a*). However, in *Nycticebus* and *Galago* (Fig. 15. 33 *b*) they are finely branched and show a distinct tendency to be polygonal. In the *Lemuridae* they attain a much more extensive development and take the form of large leaf-like folds. The mesodermal core of the villi is very vascular and the capillary vessels immediately underlie the covering epithelium, but nowhere do they indent the trophoblast cells. The chorionic epithelium remains remarkably uniform, but occasional bi-nucleate cells have been observed in *Lepilemur* (Jenkinson). Moreover, in the "zone d'implantation" in *Galago demidoffi*, it is believed to undergo differentiation into two layers, the outermost of which replaces

the denuded uterine epithelium and is variously tunnelled by the maternal capillaries (Gerard, 1929, 1932).

The microscopic structure of the mode of chorionic apposition in the Lemuroidea is of considerable interest. In *Propithecus*, *Lepilemur* and *Loris*, Strahl

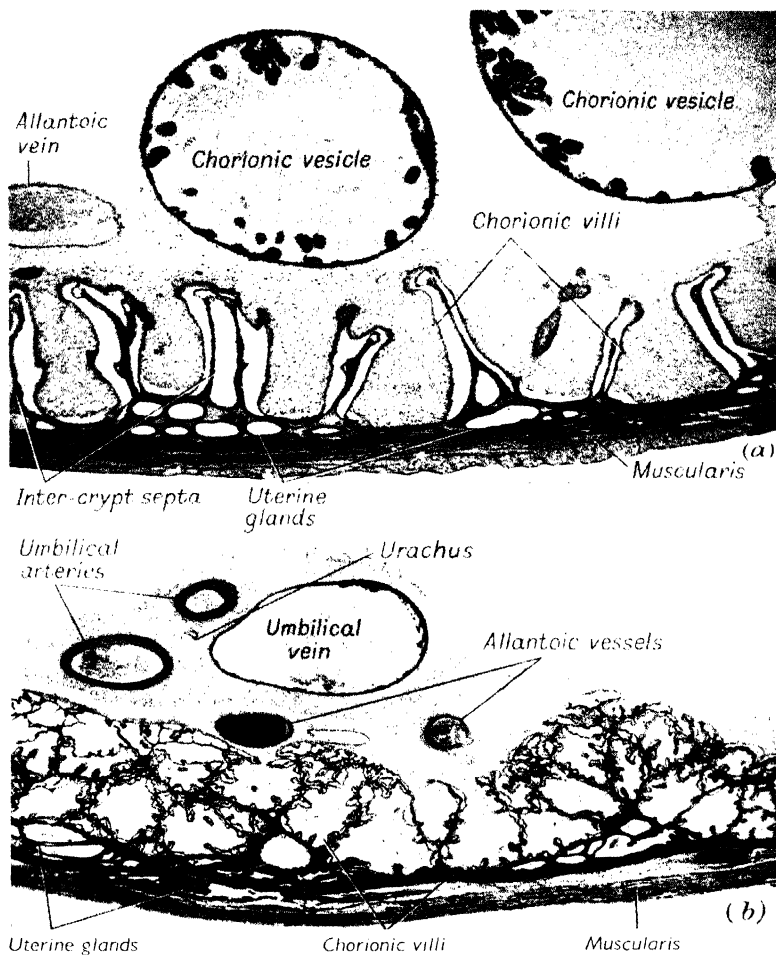


Fig. 15. 33--Sections of the placenta.

(a) *Loris*. Showing the chorion with its villi and chorionic vesicles. $\times 26$. (b) *Galago*. Showing finely branched villi and portion of the umbilical cord. $\times 13$.

(1905b), Jenkinson (1916) and Hill (1932) describe processes extending from the trophoblast cells between the uterine epithelial cells. In *Nycticebus* (Assheton, 1910) and *Chiromys* (Hill and Burne, 1922), on the other hand, the relations between uterine epithelium and trophoblast are not quite so intimate. In *Galago agisymbanus*, Strahl (1899) describes a considerable separation between the endometrium and the tips of the chorionic villi. These spaces are filled with

histotrophic material derived from the uterine epithelium. Many blood extravasations occur in the connective tissue of the mucosa, and the red blood corpuscles undergo changes as in the sheep, the glandular cells and histotrophe containing granules which give an iron reaction. At the extremity of each chorionic villus is a slight pit the cells surrounding which contain a granular greenish substance derived presumably from haemoglobin. Turner (1876b) had previously noted in Lemurs the intense brown staining of the glands from effused blood, but Hill and Burne found no extravasated blood in either *Chiromys*, *Lepilemur* or *Nycticebus*.

The allantois is large and multilobulate, involving the formation of four sacculations of the allantoic vesicle (Hill and Burne, 1922 ; Hill, Ince and Subba Rau, 1928 ; Hill, 1932). This peculiar mode of development of the Lemuroid allantois is regarded by Hill and his associates to be responsible for the early vascularisation of the entire chorion by the direct ingrowth of the umbilical vessels, following the fusion of the primary allantoic lobe with a small localised area of the chorionic surface. For a further discussion of the mode of vascularisation of the chorion in the Lemurs, see Hill (1932), and Wislocki and Van der Westhuysen (1940).

Placentation in the American Mole (Scalopus aquaticus L.)

Before concluding the present section, something must be said about the mode of development of the placenta of the American mole. Among the Insectivora the placenta is usually discoidal and of haemochorial or endotheliochorial type, but in *Scalopus aquaticus* it is of the diffuse, non-invasive variety (Mossman, 1939a) and therefore, of special interest, in view of the prominent place occupied by the Insectivora in the literature of placental phylogeny. The foetal membranes of *Scalopus* are, however, essentially similar to those of the European mole (*Talpa*), and the only fundamental difference between the two is that in the former the placenta is epitheliochorial, whereas in the latter it is endotheliochorial (Grosser, 1936).

According to Mossman's account, the placenta in *Scalopus* is very broad and thin, covering the entire lateral and mesometrial walls of the gestation sac. It is constituted simply, by the interlocking of short, moderately branched chorionic villi with uterine crypts of similar size and shape, and at parturition the villi are presumably withdrawn from their crypts without injury to either, i.e. the placenta is indeciduate. The uterine epithelium and the uterine glands persist throughout gestation in an active condition, and special "absorptive areas" which may be either flat or bowl-shaped, and may even have villous processes are differentiated opposite the openings of the uterine glands. Similar absorptive chorionic areas occur in *Talpa*. These areas seem to be exactly comparable to the initial stages in the formation of the "areolae," "chorionic vesicles" and hippomanes as observed in the pig, and certain Lemurs (Hamlett, 1935a). Moreover, as they often contain maternal blood Mossman believes that they may also be compared with the central haematoma of other Insectivora (e.g. *Centetes*) and Carnivora (e.g. Cat). However, there are other facts besides the presence of maternal blood which increases the plausibility of this hypothesis (see p. 231).

VIII. PLACENTA SYNDESMOCHORIALIS

In this type of placenta, well illustrated by the condition found in Ruminants, the uterine epithelium disappears leaving the chorionic epithelium in contact with the endometrial connective tissue. The foetal and maternal bloods are thus separated by the endothelium of the foetal capillaries, some connective tissue (not always), the chorionic epithelium, the endometrial connective tissue and the endothelium of the maternal capillaries. The situation in the placenta is, however, considerably more complex than it might appear from the above statement ; since in many so-called syndesmochorial placentae the lining of the uterine crypts may be partially or completely restored before the termination of pregnancy. The structural basis thus developed is then essentially the same as in the epithelio-chorial variety and it may be a matter of great difficulty to decide, on the basis of finer structure, to which class the placenta should be assigned. Some ruminants exhibit a combination of both types. (*See also* p. 147.)

Practically all syndesmochorial placentae are cotyledonary or multiplex, a condition which is determined by the presence from an early period, and independently of pregnancy, of numerous endometrial *caruncles* or *cotyledonary burrs*, which project as thickened knobs of the sub-epithelial tissue into the uterine lumen. During pregnancy they form connections with localised villous proliferations of the chorion. The groups of chorionic villi are known as *cotyledons*, and together with the *caruncles* form functional units that are often referred to as *placentomes* (Bonnet). Many writers, however, have enlarged the meaning of the term cotyledon to include the composite structure and employ the self-explanatory terms "maternal cotyledon" and "foetal cotyledon" to distinguish between the parts. In what follows Bonnet's term *placentome* will be used interchangeably for *cotyledon*.

The cotyledons vary in number among the different species of ruminants, ranging from about 160-180 in the goat and giraffe (*polycotyledonary* type), to 5 or 6 in the roe deer (*oligocotyledonary* type), with the sheep and cow occupying an intermediate position between the two extremes. There may be as many as 100 cotyledons in the sheep (Garrod, 1877) but more commonly they are found to vary between 88 and 96 (Ellenberger and Baum, 1921 ; Cloette, 1939 and others) about equally distributed between the pregnant and non-pregnant horns. In the cow, Rorik (1907), Somner (1912), Hammond (1927) and others, report wide variations in the total number of placentomes—70 to 142. Rorik showed that the number in the horn bearing the foetus is always in excess of that in the other horn, sometimes by as much as 150 per cent. Bergmann (1922) and Hammond (1927) also report a preponderance of functional cotyledons in the gravid horn. Adventitious cotyledons are sometimes found in places other than those of the regular cotyledons, but the presence of these is not necessarily associated with a diminished number of true cotyledons. (*See, however, Hammond, 1927.*)

Most authorities are agreed that the cotyledons are arranged in rows in the gravid and non-gravid horns. The linear arrangement is well figured in the sheep and cow by Fabricius (1600). In the sheep, Schultze (1896), Grant (1933) Cloette (1939) and others also depict four irregular rows of cotyledons. In the cow, Craig (1930) and Hammond (1927) confirm the linear arrangement and state that 4 series occupy the body, 3 the middle of the horns and 2 lie at the anterior

extremity. The linear arrangement of the foetal cotyledons on the surface of the bovine allanto-chorionic sac is illustrated in Fig. 15. 11 *b*.

The cotyledons in ruminants exhibit remarkable differences in size, form and structure, nearly related species often differing characteristically in this respect. Many of these variations seem to be of little or no physiological significance. In some animals (e.g. cow) the cotyledon is described as being convex with a distinct stalk or pedicle by which it is attached to the uterus; others (e.g. Baker's antelope) have cotyledons with flat surfaces and flat attachments, while in still others (e.g. sheep, goat) the cotyledons are concave and have broad attachments. Fig. 15. 34, which is based on Andresen's (1927) embryological studies and our own personal findings, gives an excellent idea of the form and structure of the cotyledons in a variety of ruminants. In the camel and musk deer the placenta is *acotyledonary*.

The cotyledons of ruminants have been classified according to Strahl (1912) into those exhibiting slender villi which branch at acute angles, and those which possess broad villi which branch at obtuse angles. In the latter case blood extravasations, resulting from the rupture of the uterine vessels, occur between the maternal and foetal tissues, whereas in the former no such bleeding occurs. These conclusions appear to have been accepted by Andresen. Later studies (Grosser, 1927; Krölling, 1931; and Wislocki, 1941) have shown, however, that some of the assumptions of Strahl and Andresen cannot be sustained.

In outlining the main features of the syndesmochorial condition we may take as a basis of reference the phenomena as seen in the placenta of the sheep and cow, the study of which between 1875 and 1906, laid the main basis of our knowledge of ruminant placentation and in large measure also on the typically plicate and non-deciduate variety. Both objects made classical by these pioneer researches have been repeatedly investigated by more recent observers and experimenters. They differ materially in respect to certain details of the process and hence offer a broader view when considered together.

The Foetal Membranes and Placenta of the Sheep and Cow.

The state of the endometrium before pregnancy.

As in most ruminants, the endometrium of the ewe and cow is subdivided into two parts, the uterine caruncles and the intercaruncular areas. As already indicated, the caruncles are specialised non-glandular areas to which the foetal cotyledons become attached, and are essentially local thickenings of the sub-epithelial dense connective tissue which elsewhere exists as a thin layer immediately beneath the epithelium. They are moderately well vascularised, the small blood vessels tending, on the whole, to run in a direction at right angles to the surface. In the non-pregnant ewe, the cotyledonary burrs are all more or less of the same size and are just visible to the naked eye as small circular areas on the surface of the uterine mucous membrane. In di-oestrus they are level with the surface of the mucosa, but during oestrus they appear to be somewhat more prominent. In pregnancy they become enormously enlarged, being largest near the middle of the allanto-chorionic sac opposite the dorsum of the foetus.

The epithelium lining the cavity of the uterus consists of a single layer of tall columnar cells, rather taller over the burrs than elsewhere. The nuclei of these cells are basal in position and not always at the same level, a condition that may be

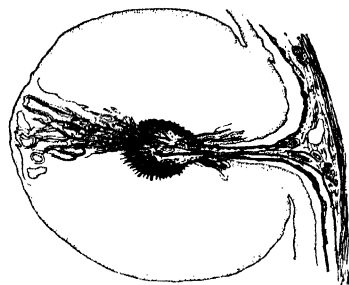
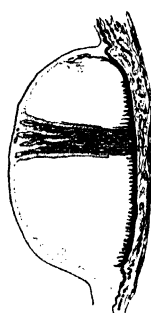
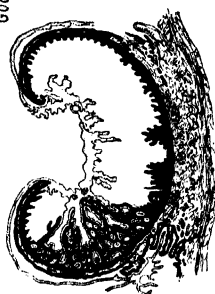
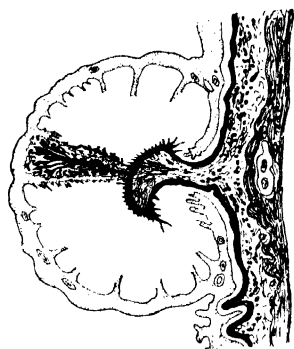
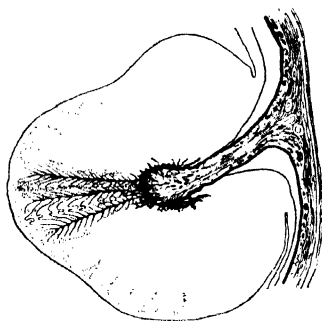
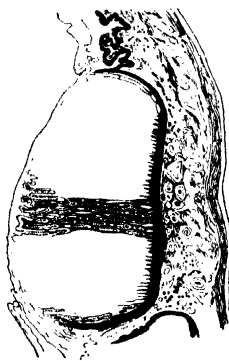
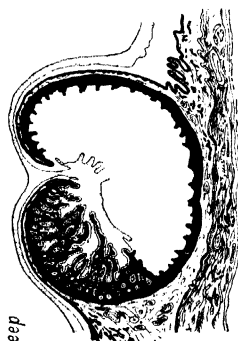
Roe Deer*Red Deer**Goat**Cow**Baker's Antelope**Indian Antelope**Red Brocket**Reed Buck**Sheep*

Fig. 15. 34—Diagrams to show the gross form of the placentomes (cotyledons) in a number of ruminants.

responsible for the statement that the epithelium is stratified. Cilia are usually absent in the sexually mature non-pregnant uterus, but their presence has been reported, especially in young animals (Ellenberger and Baum, 1921). However, all trace of ciliation disappears with the onset of pregnancy. In the intercaruncular areas the epithelium dips down at intervals into the lamina propria and forms long tubular glands which are narrow, much coiled, and highly branched at their base; towards the cervix the number of glands decreases.

A striking feature of the uterine mucosa is the presence, in some species, of black pigment. According to Grant (1933), the characteristic pigmentation of the uterine mucosa and oviducts of the ewe is due to the presence of true melanoblasts similar to those found in the skin and other situations and not to pigment of haematogenous origin, as was suggested by Bonnet (1880, 1882a) and others,

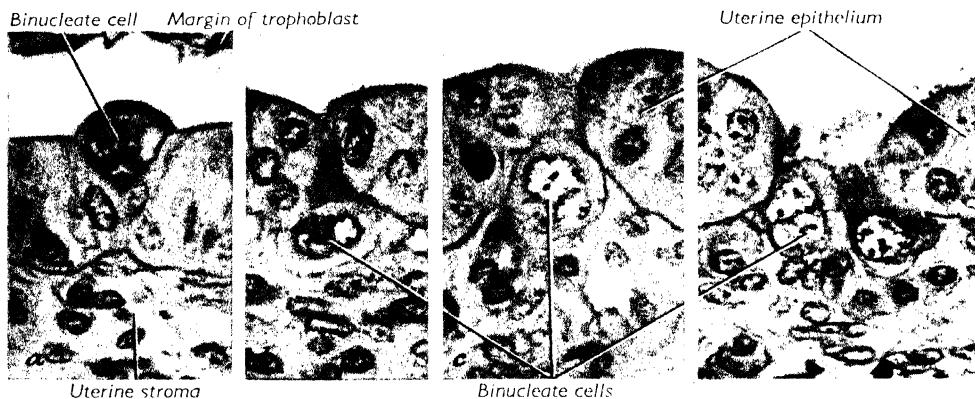


Fig. 15.35—Stages in the penetration of the uterine epithelium by the binucleate cells of the trophoblast from an ewe 20 days pregnant. 600.

(a) A binucleate cell has just left the trophoblast and is forcing its way as a wedge between the uterine epithelial cells. (b) Arrival of a cell at the base of the epithelium. (c) The invading cell hypertrophies at the base of the epithelium and separates it from the underlying connective tissue of the burr. (d) Degenerating epithelium separated by two trophoblast cells.

and neither the occurrence of pigmentation nor its intensity bear any relation to the stages of reproductive activity. Pigment granules may sometimes be found in the uteri of foetal lambs.

Preimplantation phenomena.

Certain changes occur in the caruncles and the intercaruncular areas before attachment. In the intercaruncular regions they are confined for the most part to the uterine glands. The leucocytes, chiefly of the lymphocyte type, which in the non-pregnant uterus are situated at the base of the lining epithelium, increase in number and wander out between the epithelial cells mingling with the secretion lying in the uterine cavity. There is considerable oedema and increased vascularity in both the caruncular and intercaruncular areas; in the former the stromal connective tissue is more densely cellular and more vascular than it is elsewhere. In the intercaruncular areas the glands show evidence of growth. They become more coiled and their cells increase in height;

the glandular sacs, situated at the junction of the branches with the main ducts, expand greatly and actively secrete.

Implantation.

The mode of attachment of foetal and maternal cotyledons in the ewe has been studied by Assheton (1906) and personal findings are in agreement with his. It is generally held that the surface epithelium is not destroyed, but Assheton has shown that on the cotyledonary burrs it is distinctly degenerated by the seventeenth day, and he has brought forward strong evidence that it is not subsequently regenerated but is replaced by bi-nucleate cells of the foetal ectoderm. In the sheep the first attack upon the maternal uterine mucosa occurs between the seventeenth and eighteenth day after service. At this time there is a very loose attachment of membrane to caruncle, the former being held in position by the accumulation of fluid in the central portion in much the same way as the inner tube of a pneumatic tyre is held in the cover (Assheton, 1906). At about the 31st day villi appear on the surface of the chorionic sac, resulting in closer attachment. At the 44th day the foetal cotyledons are visibly arranged in rows, and have convex free surfaces which fit into the concavities of the maternal cotyledons. By this stage there is a fair degree of adherence between the maternal and foetal parts. By the 78th day the general features of the ripe placenta are apparent and from now on the edges of the maternal cotyledons curl inwards to hold the foetal cotyledons in position, thus completing the cotyledonary attachment (*see* Fig. 15. 40 *a*).

In the cow, Hammond (1927) has given a careful and accurate account of the histological changes which occur in the cotyledons during the course of pregnancy. He has reported that attachment does not begin until the end of the first month and is not completed till sometime during the third or even fourth month. Before attachment is effected, the uterine epithelium covering the surface and base of the cotyledonary burrs is completely denuded. By the end of the second month there is a fair degree of adherence between the chorionic villi and the interlocking lamellae of the maternal cotyledons. At three months a large and more complete system of branching in both the maternal and foetal projections occurs and separation is not easily effected. The appearance and relations of the parts in the ripe cotyledon are shown in Fig. 15. 40 *b*.

The destruction of the epithelium is brought about at first by the agency of darkly staining binucleate cells of the trophoblast. These can first be recognised deep down in the foetal ectoderm, whence they migrate through the maternal epithelium and force themselves between the lining cells and the underlying stroma. Fig. 15. 35 gives an excellent idea of the typical shape and relationships of the binucleate cells in the ewe. In their passage they become enormously enlarged and give rise to a layer of cells which seem to enter into very close protoplasmic relations with the cells of the stroma. In the destruction of the epithelium they are aided by others which are more phagocytic, and which attacking from the surface actually engulf portions of the uterine epithelium (Figs. 15. 36 *a, b*). By the twentieth day the whole surface of the burr is denuded of epithelium, its place being taken by an irregular and intermittent layer of enlarged trophoblast cells. Sometime later this layer is quite continuous (Fig. 15. 36 *c*), but with the advance of pregnancy it again becomes lost at certain places (e.g. at the tips of the maternal septa).

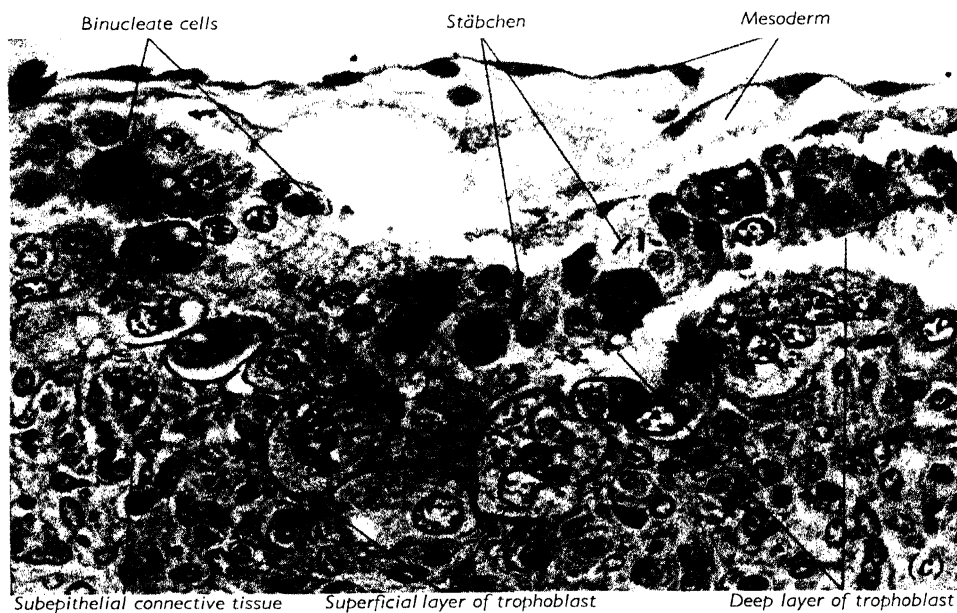
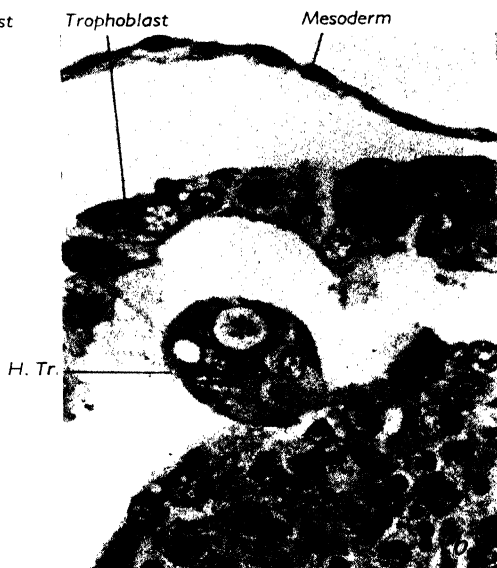


Fig. 15. 36—Stages in the destruction of the uterine epithelium by the trophoblast. From the same ewe as in Fig. 15. 35. $\times 500$.

(a) Two trophoblast cells are seen on the surface of the epithelium. The larger with a distinct vacuole is engulfing two uterine epithelial cells. The smaller contains a lightly staining epithelial cell undergoing destruction. (b) An hypertrophied trophoblast cell (H. Tr.) has just left the trophoblast and is attacking the few remaining epithelial cells on the surface of the burr. Three disintegrating epithelial cells, with lightly staining nuclei, are seen within it. (c) The uterine epithelium has now been completely removed from the surface of the burr. The trophoblast is differentiated into two layers. The superficial layer consists of a continuous stratum of discrete, hypertrophied, multinucleate cells, whereas the deeper layer contains smaller, darkly staining binucleate cells as well as ordinary trophoblast cells.

Where the trophoblast is in contact with the surface of the burr it is greatly thickened and the cells are filled with rod-like bodies, the "Uterinstäbchen" of Bonnet. Later they appear in the uterine milk.

In the inter-cotyledonary areas the contact of the foetal trophoblast with the uterine mucosa also results in the erosion of the uterine epithelium. Here large globular cells occur over the abraded surface just as they do over the burrs, and the evidence is all in favour of these cells being of foetal and not maternal origin, as some writers suppose. This denudation of the epithelium is more widespread in the ewe than in the cow and persists until midway through the fourth month of pregnancy, after which the epithelial lining appears to be restored. In this

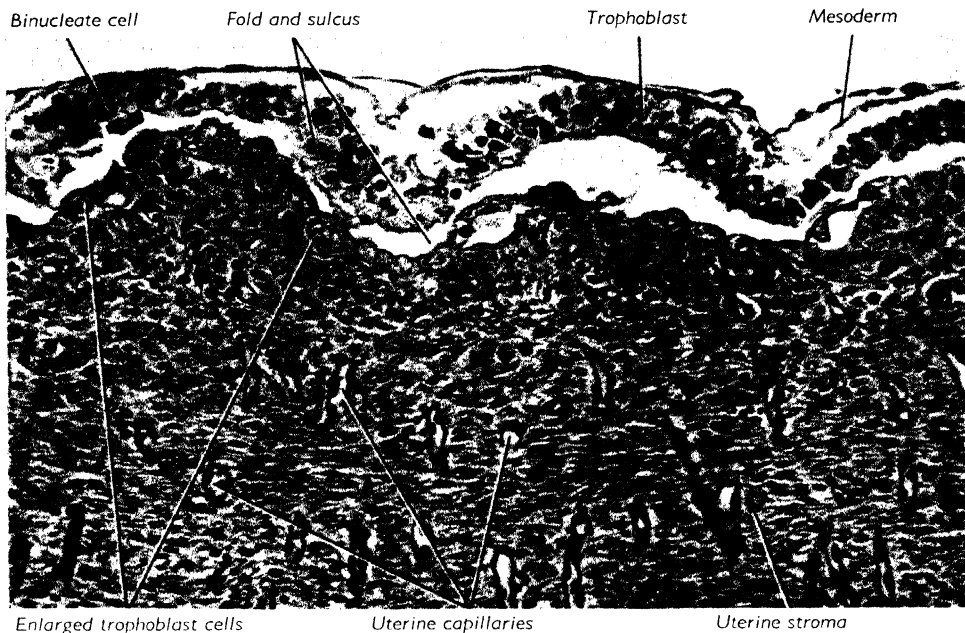


Fig. 15. 37.—Section through the uterine and embryonic parts of a placentome of the sheep at the twentieth day of pregnancy. Folds of the trophoblast fitting into the sulci of the cotyledonary burr. $\times 210$.

connection it is noteworthy that the uterine epithelium always persists around the openings of the uterine glands, which are wholly intercotyledonary in position, whence we may presume that the glandular secretion, in preventing the close adherence of the trophoblast at these points, shields the glandular epithelium from the phagocytic action of the binucleates. In this region there is no formation of a spongy layer in the sub-epithelial tissue as in the burrs. But a great change occurs in the glands; they increase in length and complexity, and secrete actively.

Formation of villi and crypts.—In the cotyledonary areas of the trophoblast, villi are developed as buds of the foetal ectoderm (Fig. 15. 37), which afterwards contain cores of mesoderm with branches of the allantoic vessels (Fig. 15. 38). In the vicinity of the commencing villi and marking the intervillous areas, the trophoblast assumes an "arcade" like structure, which seems to form the foundations

from which the villi spring. The villi fit into depressions or crypts on the surface of the maternal cotyledons, increase in length, and branch in different directions as pregnancy advances (Fig. 15. 39 *a*). Whether they literally grow into the maternal



Fig. 15. 38—Section through foetal villi and uterine crypts of the sheep at the thirty-fifth day of pregnancy. The villi show a core of loose mesenchyme covered by a layer of ordinary trophoblast cells with an occasional, darkly staining binucleate cell. The crypts and the surface of the intercrypt columns are lined by a layer of darkly staining binucleate cells. Note the axial blood vessel in the mesenchymal stroma of the villus. $\times 100$.

tissues either mechanically or by a phagocytic action is uncertain. In the sheep, Assheton did not observe any engulfment of cells after the uterine epithelium was denuded. It seems more likely that very little, if any, further penetration occurs, but that the subepithelial tissue swells and keeps pace with the villi as they increase in length. The cotyledonary crypts are lined with foetal ectoderm and are not secretory in the true sense of the word.

The subepithelial tissue is represented in the non-pregnant uterus by a thin layer of dense connective tissue with localised thickenings in the burrs. With the onset of pregnancy occurs an infiltration of lymph between the more superficial cells and the subepithelial layer, and an increase in the number and size of the blood capillaries and lymphatics. Thus the layer becomes spongy and hypertrophies around the foetal villi, producing the cotyledonary interdigitations, the main bulk of which consists of the material of the fibrocellular zone together with the included capillaries. In the cow, Jenkinson (1913) states that the crypts arise as downgrowths from the maternal epithelium into the subepithelial layer. Hammond (1927), however, does not agree with this since he considered it extremely unlikely that the tro-

phoblast cells, which Jenkinson himself admits are phagocytic, would fail to absorb the uterine epithelial cells as had been shown to occur by Assheton in the sheep (*see also* Figs. 15. 36 *a, b*). Hammond believes that the crypts arise as outgrowths of the subepithelial connective tissue of the burr; it is from this layer that adventitious cotyledons are formed in the intercotyledonary regions.

The histology of the cotyledons.

The fundamentally important questions of reorganisation of the growing cotyledon and the minute structure of the "placental membrane" must now be examined more closely. Many controverted points are here involved and a final evaluation of the many opinions must await a fuller knowledge of the histology of the placenta in more of the many representatives of the ruminants. Nevertheless, it seems necessary to offer some sort of provisional grouping of the facts. The chief questions at issue centre around the extent of the denudation of the uterine epithelium and the origin of the lining cells of the maternal crypts. In

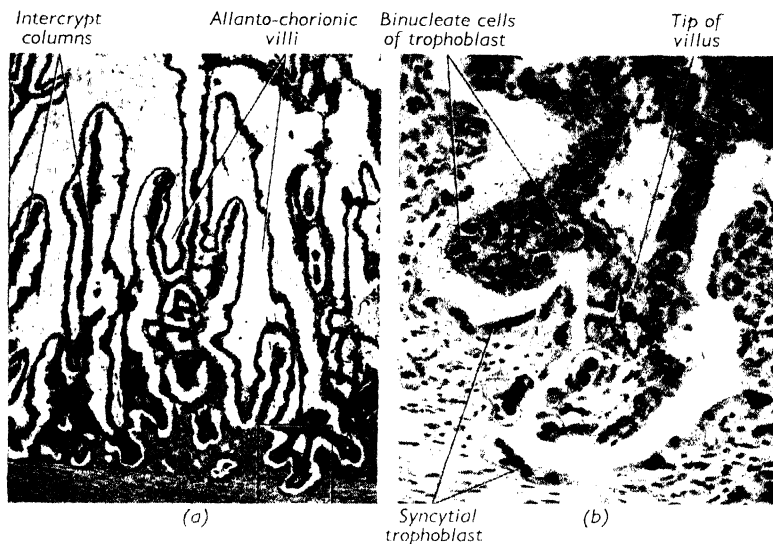


Fig. 15. 39.—Giant cells of crypt walls.
(a) Vertical section through the inner part of a placental crypt of the sheep at the sixty-fifth day of pregnancy. The interlocking of the foetal and maternal tissues is well shown. Area enclosed in rectangle is shown at higher magnification in Fig. 15. 39 (b). $\times 30$. (b) Detail of area enclosed in rectangle in Fig. 15. 39 (a), showing binucleate cells in trophoblastic covering of villus, and enlarged trophoblast cells lining fundus of crypt. $\times 160$.

other words, is the ruminant placenta wholly a syndesmochorial one, or is it in part an epitheliochorial one? Among all the ideas put forward the most suggestive, in view of what has more recently been ascertained, is that of Assheton (1906), namely, that the epithelium of the crypts is completely removed and is replaced by cells of the foetal trophoblast. The nature of the foregoing process has been re-examined in the sheep, goat, cow and black-buck (*Antelope cervicapra*) and it is consequently with them that the following discussion will chiefly deal.

Figs. 15. 40 a, b, c, d, illustrate the microscopic structure of the ripe cotyledon in a series of more or less typical cases. The free surface of the cotyledon is deeply concave in the sheep and goat—less so in the blackbuck—but markedly convex in the cow, where also the base of attachment is constricted to a narrow pedicle. The cotyledon consists of numerous fairly slender, but richly vascular chorionic villi which are accommodated in furrows or crypts of the uterine

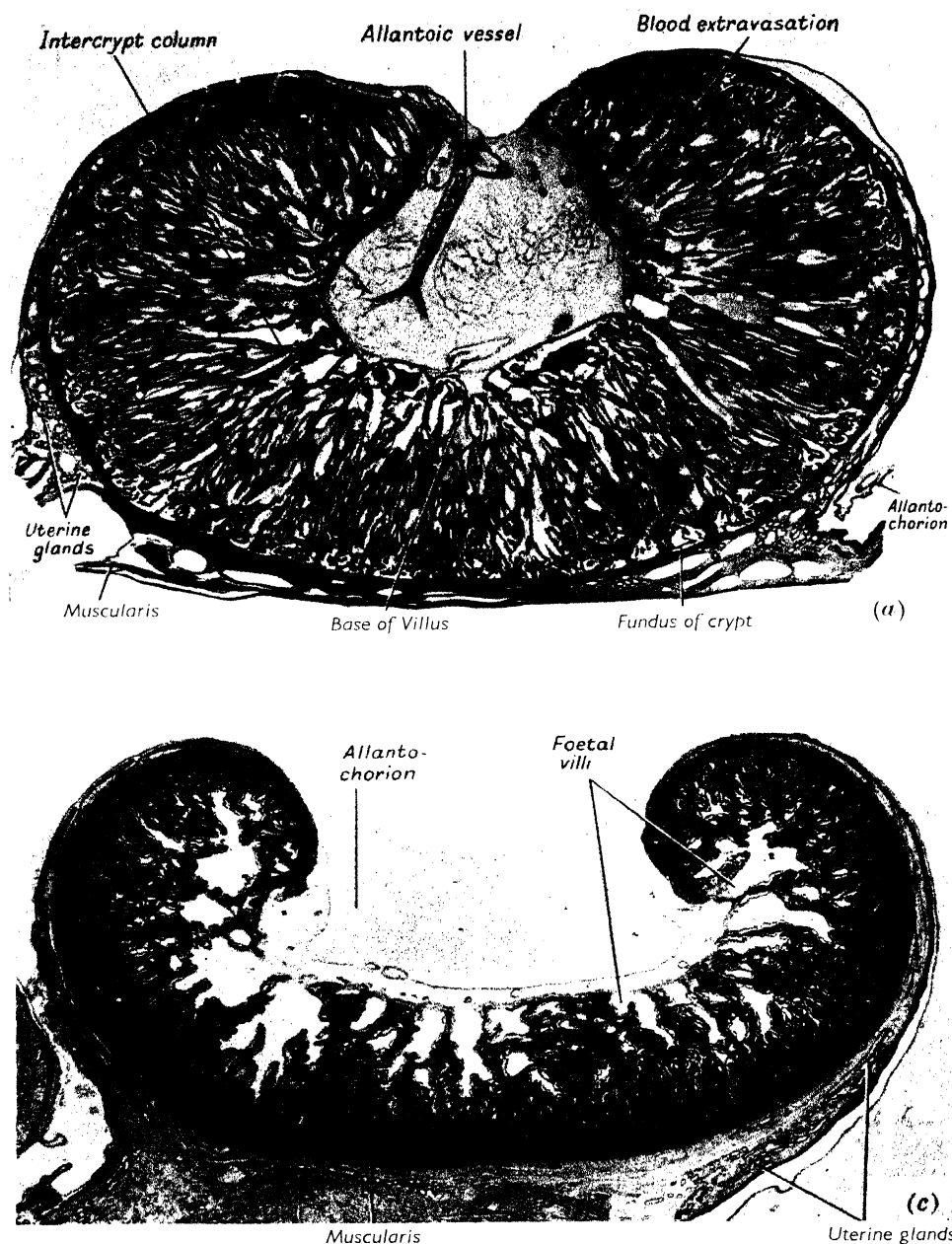
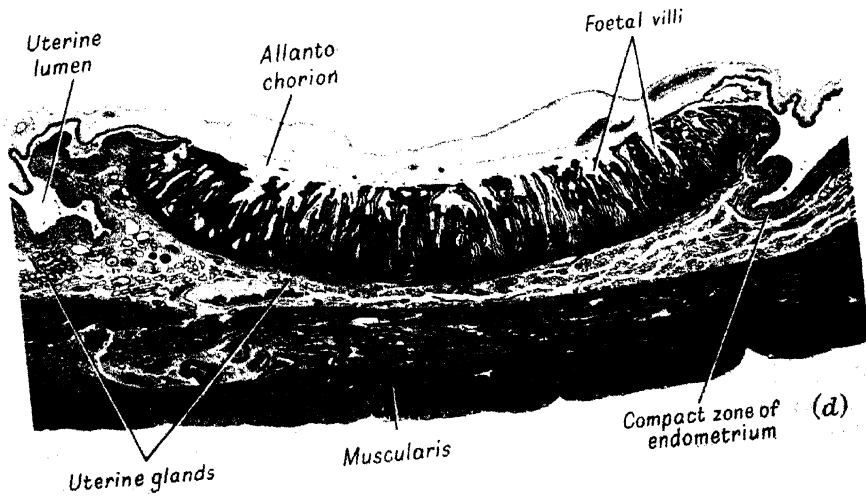
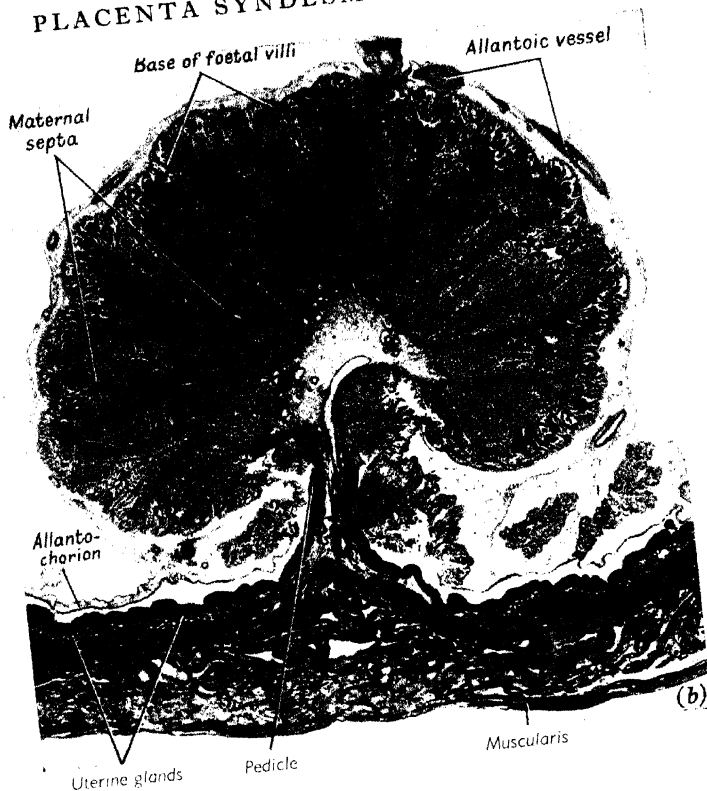


Fig. 15. 40.—Photomicrographs of vertical sections of various types of placentomes. (a) From a sheep at the 100th day of pregnancy. $\times 4$. (b) From a cow 90 days pregnant. $\times 6\frac{1}{2}$. (c) From a goat 60 days pregnant. $\times 11$. (d) From an Indian antelope (Foetus; G.L. 12 cm.). $\times 13$.

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PLACENTA SYNDESMOCHORIALIS

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mucosa. Near the surface of the cotyledon the villi are irregularly branched, whereas in the depths of the crypts they tend to be arranged in parallel rows at right angles to the surface of the cotyledon. Each villus is composed of a core of richly vascular mesoderm and is covered by a layer of cuboidal or polyhedral trophoblast cells, many of which are of the binucleate variety (Fig. 15. 39 *b*). At the bases of the villi the trophoblast is very tall and columnar. The mesoderm is made up of loose gelatinous Wharton's Jelly carrying the axial blood vessels, while numerous small capillaries ramify between it and the covering trophoblast.



Fig. 15. 41—Portions of two foetal villi and the intervening inter-crypt column of a red deer (Foetus: G. L. 6 cm.), showing the nature of the epithelial investment of the maternal septum. Here the trophoblast is differentiated into two layers each containing a number of binucleate cells. On the left of the figure the two layers are separated by an artificial space; on the right they form a uniform membrane. $\times 390$.

In the intercotyledonary region chorionic villi are absent. Here the trophoblast is lined with the same type of epithelium as on the surface of the villi, but in many places raised, circular, and richly vascular areolae resembling those on the pig's allanto-chorion mark the openings of the uterine glands. They are more prominent in the sheep than in the cow and act as centres of absorption of materials elaborated by the uterine glands (v. Baer, 1828; Turner, 1875; and Assheton, 1906).

Mention has already been made of the maternal tissue which forms the wall of the crypts in which the chorionic villi are located. These *intercrypt columns* or *maternal septa* are somewhat club-shaped and are composed of rather cellular and well-vascularised connective tissue. In the sheep, goat, blackbuck and red deer, their surfaces are clothed by a layer of irregular cells which is loosely attached at places but firmly fused at others to the uterine wall (Fig. 15. 41). This covering epithelium is thickest in the goat, while in the cow it takes the form of a low, cuboidal, cellular epithelium (Fig. 15. 42). Binucleate cells are abundant; they are identical with those on the villi and like these contain abundant

stores of alkaline phosphatase. At certain points, especially at the fundus of the crypts (Fig. 15. 39 *b*), the lining cells become syncytial and fuse with the subjacent stroma, the larger masses of syncytiotrophoblast being often in close connection with the blood capillaries, at least in the early stages of the formation of the cotyledonary interdigitations. At the tips of the maternal septa the lining cells are less obvious. In the full term cotyledon binucleate cells are fewer in the villi but more numerous in the walls of the crypts; the villi and the maternal trabeculae are filamentous and, in both, there is very little connective tissue. At this stage the amount of adherence between the uterus and the foetal membranes is slight and they may be separated with ease. As a general rule the entire foetal cotyledon comes away at parturition, but in the contra-deciduate sheep trophoblast cells

adhere to the maternal cotyledon, which is now attached to the uterine wall merely by a thin mucosal pedicle.

At the apices of the intercrypt columns extravasations of maternal blood are formed by repeated haemorrhages from the superficial capillaries. In the sheep, Assheton (1906) states that he found them in all the cotyledons from the 75th day of pregnancy onwards. At about the end of the third month the cotyledons appear to reach their maximum development. From this time until term (150 days) the extravasated maternal blood accumulates in the uterine spaces at the bases of the villi and forms lacunae into which the foetal trophoblast hangs. The character of the trophoblast has changed somewhat; the superficial parts of the cells are vacuolated and Jenkinson (1906) has shown that the red blood corpuscles are absorbed by the phagocytic action of these cells. In the cow blood extravasations are apparently not a constant phenomenon. The accumulation of maternal red corpuscles in the spaces between the bases of the foetal villi and apices of the maternal septa is figured by Fraenkel (1898) and Hammond (1927). Hammond states that the accumulation of blood is very marked after the 5th month. In the deer, blood is effused into the glands, but no extravasations take place in the cotyledons (Strahl, 1906b).

The nature and origin of the investing tissue of the maternal septa have proved to be exceedingly difficult and controversial. By Assheton (1906) the lining epithelium of the ovine crypts were believed to be a foetal plasmodium formed by the fusion of originally separate cells. He states that these lining elements are the same as the irregular lining which was found at the close of the attack upon the uterine epithelium by the foetal trophoblast. This conclusion is, we believe, well founded.

Since the researches of Assheton, the opinion has gained ground that the tissue investing the maternal trabeculae arises by direct transformation of the maternal mucosal elements; but which specific cells of the mucosa give rise to the lining cells is not yet decisively demonstrated. In the cow, Kolster (1902), Ledermann (1903), Jenkinson (1906), Pomayer (1919) and Chiodi (1927) believe



Fig. 15. 42—Transverse section through the middle of a cotyledon of a cow 3 months pregnant, showing the stages in the replacement of the uterine epithelium by the binucleate cells of the trophoblast. *a, b, c* and *d*, binucleate cells in epithelium covering tips of villi; *e, f*, two binucleate cells which are partially detached from villus; *g, h*, two invading cells which have now reached the wall of the crypt. $\times 500$.

them to be modified uterine epithelium. Hammond (1927), on the other hand, states that they are not true epithelial cells, "but consist of connective tissue, plasma or lamella cells whose function is nutritional and which tend to congregate on the abraded surface." Andresen (1927) who describes a junctional zone in the cotyledons of various ruminants, ascribes to the syncytial masses, including the giant cells an origin from the trophoblast covering the villi, but with this Wislocki (1941) is unable to agree, although he is himself doubtful from which specific cells of the mucosa they arise. Their origin from mucosal elements Wislocki regards as indicative of a reaction similar in character to the decidual reaction encountered in placentae of the typically deciduate variety.

It is clear from the foregoing that much uncertainty still remains regarding the nature of the lining cells. Nevertheless, the observations recorded in this section raise anew the possibility that these cells, in some species at least, are epithelial and of trophoblastic origin as Assheton supposed, instead of being derived from the uterine epithelial or connective tissues as Pomayer, Kolster, Hammond, Wislocki and others have indicated. The accompanying illustrations provide ample evidence of the direct passage of binucleate cells from the trophoblast to the surface of the maternal endometrium, to become, in some cases (e.g. sheep), typical syncytial giant cells (Fig. 15. 39 *b*) ; in others (e.g. cow), simple cuboidal elements (Fig. 15. 42). Moreover, in the cow and to a lesser extent in the goat, the replacement of the epithelial investment of the mucosal crypts results in the formation of what appears to be an epitheliochorial relationship not unlike that of the horse (cf. Figs. 15. 29 and 42). Nevertheless, it is quite clear, also, as is true of the placenta of the sheep and blackbuck, that the cells lining the crypts are foetal, and trophoblastic in origin. Consequently, the "placental membrane," according to strict definition, and employing Grosser's terminology, is of the syndesmochorial rather than the epitheliochorial type.

Growth of the cotyledons.

The current notions of rapid growth of the placenta during the first part of pregnancy, followed by a period of little development, are supported by the figures given for the goat by Elliott, Hall and Huggett (1934) and Barcroft *et al.* (1934), and for the sheep by Cloette (1939) and Barcroft (1946) as well as by personal findings. In these species (Table V) placental weight is shown to increase at an accelerating rate to the 13th week of pregnancy after which there is an abrupt change in the growth of the organ ; not only does it stop growing but it diminishes progressively in size throughout the remaining part of gestation. As will be mentioned in another connection this variation in weight of the placenta is affected by the change in size of each individual cotyledon.

The course of the blood flow in the sheep's placenta.

Recently the vascular relations in the sheep's placenta have been worked out by Barcroft and Barron (1946). They found that the foetal and maternal capillary streams, instead of coursing parallel to one another actually flow in opposite directions. The picture in the case of the cow's placenta is essentially similar. In the sheep (Fig. 15. 43), when the chorionic villi are well developed (between the 50th and 80th days), each is supplied by an axial artery which runs in the abundant

TABLE V (From Cloette, 1939)

Class.	No. of Ewes.	Mean Weight of Placenta.	Mean Diameter of Cotyledons.		Mean Height of Cotyledons.	
			Pregnant Side.	Non Pregnant Side.	Pregnant Side.	Non Pregnant Side.
			cm.	cm.	cm.	cm.
Non Pregnant	11	nil	0.28	0.30	0.15	0.16
1st Month .	12	?	0.42	0.39	0.16	0.15
2nd Month .	8	?	.86	.47	0.63	0.55
3rd Month .	6	519 gm.	.45	.24	1.42	1.37
4th Month .	7	349 gm.	.04	.94	1.50	1.46
5th Month .	5	307 gm.	3.00	.00	1.16	1.16
Average .			1.63	.52	0.681	.656

Wharton's Jelly direct to the tip of the villus. Here it breaks up into a rich capillary plexus which continues back towards the foetus in close relation to the chorionic epithelium. At the base of the villus the fine meshed capillaries unite to form tributaries to veins which collect blood from the adjacent portions of the neighbouring cotyledons. The apex of the villus has the fine mesh arranged parallel to the surface epithelium, the average diameter of the capillaries ranging from 9 to 12 μ .

The exact nature of the changes in the cores of the chorionic villi has also been studied. According to Barron (1945), there is a rapid development of the masses of Wharton's Jelly in the villi between the seventh and eleventh weeks followed by a shrinking from the twelfth to the fourteenth week, so that as embryonic development progresses the masses tend to disappear leaving their vascular coverings behind. Accordingly, the density of the vessels on the surface and beneath the trophoblast in a single standard section of the cotyledon constantly rises, while the diameters of the individual villi correspondingly fall. The implications of these relationships are considered in detail in Chapter 17 on Foetal Respiration.

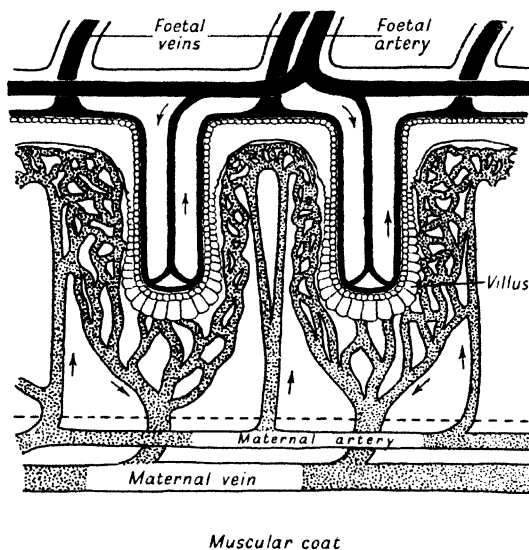


Fig. 15. 43—Arrangement of blood vessels in the placentome of the sheep's placenta. (Redrawn and slightly modified after Barron.)

Before leaving the subject of the blood supply of the chorionic villi, it is necessary to say something further about the completeness of the capillary network which supplies them in certain antelopes. For example, in *Madoqua (Rhyhcho-tragus) kirkii* investigated by Wislocki (1941) and in the black-buck (*Antilope cervicapra*) the cytotrophoblast is tunnelled with intra-epithelial capillaries (Fig. 15. 44), so that the intimacy of the maternal and foetal circulations at these points is very striking—a structural arrangement which seems to favour gaseous exchange.



Fig. 15. 44—Indian antelope (*Antilope cervicapra*). Section through the apex of an intercrypt column and portions of two adjacent villi, showing intraepithelial capillaries in trophoblastic covering of villus. $\times 530$.

In contrast to the foetal vessels, the maternal ones permeate the whole tissue of the mucosal trabeculae. The uterine arteries feed the intercrypt columns by arterial branches which pass directly to the tips of the maternal septa, where they break up into capillaries. From this capillary net the blood returns to the maternal side of the placenta, to reach the veins, through a network of tortuous channels at the base of the crypt, formed by anastomosing vessels from neighbouring columns. In the intercrypt columns the maternal capillaries are more dilated than outside the burrs, and they come close up to the surface, some of them even impinging on the lining membrane of the crypts. Except at the tips of the maternal septa, where small haemorrhages occur, these vessels withstand successfully the process of erosion. Between them and the allantoic vessels in the villi intervene only a small amount of mesoderm (not always), the cellular trophoblast, and the lining of the crypts which, according to Assheton, corresponds to the syncytio-trophoblast of the deciduate types of placentae. If, therefore, we accept Assheton's conclusions—convincing evidence of which is offered in the figures illustrating this section—that the lining cells of the crypts are of foetal, and not maternal, ancestry, it must be admitted that the placenta in the cotyledons of the sheep, goat, cow, and black-buck, in that it consists of a layer of trophoblast closely applied to intact

maternal vessels, bears a closer resemblance to that of carnivores (endotheliochorial) than to any other group of animals. In apparent harmony with this conception are the experimental results of Pohl, Flexner and Gellhorn (1941) who find, that in the placenta of the goat, the "rate of transfer of radio-active sodium per gram of placenta is of the same order of magnitude as that of the endotheliochorial placenta of the cat at comparable stages of pregnancy."

Foetal membranes and fluids.

The formation and appearance of the foetal membranes of the sheep and cow

are well described by Bonnet (1889b), Assheton (1906), Zietzschmann (1924), Hammond (1927) and others.

Membranes.—Rather large at first and provided with an area vasculosa, the yolk-sac is rapidly outgrown by the allantois. By the thirteenth day in the sheep one-third of its circumference is surrounded by coelom, and in this area the yolk-sac is separated from the chorion. At the seventeenth day the separation of the yolk-sac is complete all round (Bonnet). It continues, however, to grow *pari passu* with the blastodermic vesicle, and is gradually pushed to one side by the enlargement of the coelom. At the twenty-fifth day it is reduced to a solid rod of cells with a few blood vessels on its outer surface and it disappears before the end of pregnancy (Assheton). The vitelline circulation is initiated before the yolk-sac severs its connection with the chorion, so that a chorio-vitelline placenta is established and remains functional till about the seventeenth day of pregnancy.

Like Assheton (sheep) and Hammond (cow), Zietzschmann states that early in pregnancy the allanto-chorion increases rapidly and by the fourth (sheep) or fifth (cow) week comes to occupy both horns of the uterus. At this time the sac is distended with fluid except at its tip ends, which are attenuated like the early stages in the pig. The relation of the amnion to the chorion is quite similar in the cow and in comparable stages in the sheep; in both species an extensive portion of the amnion becomes apposed and adherent to the chorion with complete obliteration of the exo-coelom. The relationship of the amniotic and allantoic sacs and of the chorion are shown in Fig. 15. 11 b. (See also Fig. 15. 45).

The topography of the allantois in the cow and sheep is quite different from the large allantois which envelops the amnion in the horse. The relation of the allantois to the amnion in the cow after the chorion had been partially dissected away is shown in Fig. 15. 45. The interesting feature here is the relatively restricted extent of the allantois which is in contact with the amnion; another is that the allantoic sac is divided into two compartments which communicate with each other and with the urachus by means of a narrow tube-like cavity. Obviously this topographical relationship is quite different from the condition shown by Mossman (1937) and by Hamilton, Boyd and Mossman (1946) who portray all Perissodactyla and Artiodactyla as alike in possessing an allantois which envelops the amnion. That such a generalisation is correct seems very doubtful; for though the allantois attains enormous dimensions in the cow, sheep, goat and pig it by no means envelops the amnion in the manner of the horse. An even greater restriction of the allantois has been recorded in Antelopes (*Rhynchotragus*, *Antelope cervicapra*) and in members of the two families *Tragulidae* and *Cervidae*.

Another point of difference resulting from the disposition of the allantois, pertains to the divisions of the umbilical cord. In contrast to the Perissodactyla (horse, tapir), the cord in Artiodactyla (cow, sheep, goat, pig) consists of only an amniotic portion. Zietzschmann (1924) said that the bovine cord was relatively short and gave its length as between 30 and 36 cm.; similar figures are given for this animal by Williams (1940). Craig (1930), however, gave a range of 22·5 to 45 cm. For the sheep, Franklin (1944) gives a range of 20–25 cm. Like many other mammals the cord in these animals is vascularised by small blood vessels which are branches of the umbilical vessels; nerve bundles are present in the bovine cord. As in the horse there is a well developed sphincter muscle. A detailed consideration of all information bearing upon these subjects cannot be given

here ; and once more the reader must turn to Franklin's (1944) more extensive review.

The necrosis of the ends of the membranes which Jenkinson (1913) believed to be the result of a restricted blood supply is not so marked in the cow as it is in the sheep and pig ; a circumstance which may, according to Hammond (1927), facilitate the fusion of neighbouring chorionic sacs in twin pregnancies, thus increasing the frequency of free-martins in cattle as compared with sheep or pigs (see, however, page 165 and Ewen and Hummason, 1947).



Fig. 15. 45—Photograph of cow foetus removed from the uterus at the 90th day of pregnancy with the chorion partially dissected away to show the relations of the allantois. $\frac{2}{3}$ natural size.

A free-martin arises when the foetal membranes of male and female embryos become united in the uterus, so that an anastomosis of the chorionic blood vessels is established and the female is modified in the male direction. When both members of calf twins are of the same sex, a free-martin is never known to occur. So essential is the mingling of the bloods for the production of the free-martin condition that in heterosexual cattle twins in which no vascular anastomosis occur, there is no alteration of the gonads and accessories of the female. Because the sex reversing effect is limited to the production of male characters in a zygotic female, it has been regarded as the specific action of a hormone elaborated by the male foetus (Lillie, 1916, 1917 ; Keller and Tandler, 1916). For an analysis of the

free-martin condition the reader is referred to Willier's and Witschi's articles in Allen's "Sex and Internal Secretions" (1939) and to the review by Swett, Matthews and Graves (1940).

The weights of the sheep's membranes at different stages of pregnancy are recorded by Malan and Curson (1937) and by Cloette (1939). Rorik (1907) and Hammond (1927) give figures for cattle. From these sources it is seen that the foetal membranes show a different course of development from the placenta. The total weight of the membranes gradually increases during the whole of the gestation period, although like the maternal part of the placenta it first increases and then decreases as a percentage of the weight of the whole conceptus. In addition, Cloette's figures show that just prior to parturition the membranes have attained more than 27 times their weight at the end of the first month of pregnancy, whereas in the cow the increase is more than 1,500 times (Hammond).

Fluids.—Most of the available data on the foetal fluids in ruminants are summarised by Needham (1931). Additional data for the sheep will be found in the papers of Malan and Curson (1937), Cloette (1939), and for the cow in Hammond's (1927) monograph. These data for both species show that the quantity of foetal fluid increases rapidly during the first half of pregnancy, while later the increase is much less. This increase is not shared equally by the two fluids, what increase does occur during the second half of pregnancy concerns mainly the allantoic fluid. Considered as a percentage of the weight of the gravid uterus, the sum of the two fluids decreases with the approach of parturition. The amniotic fluid, however, first increases and then decreases as a percentage of the weight of the whole conceptus, while the behaviour of the allantoic fluid is just the reverse, showing first a decrease and then an increase (Malan and Curson). Hammond also draws attention to the fact that cessation of increase of the foetal fluids coincides with the inception of secretory activity of the mammary gland. He suggests that this may be significant and no mere coincidence. In the cow, the volume of the amniotic fluid at term varies from 1.1 to 4 litres (Zietzschmann, 1924); Williams (1940) said from 5 to 8 litres. For the allantoic fluid Zietzschmann gives a range of 3.5 to 12 litres. In the sheep near term Cloette gives the mean volume of fluid of 762 ml. for the allantoic and 329 ml. for the amniotic. The presence of "hippomanes" in the allantoic fluid has been already referred to. (See p. 185.)

Uterine milk.

That the uterine secretion which bathes the surface of the membranes is absorbed by the foetus and that it may even serve some useful function during intrauterine life is not a new idea. The existence of a nutritive juice in the uterus of ruminants during pregnancy has been recognised since the days of William Harvey. He spoke of an albuminous fluid, which might be squeezed out from the cotyledons of the placenta, as a source of nutriment for the foetus. As to its origin, he says in one of his letters (1655): "It might be transported by the uterine arteries and distilled into the uterus." The secretion of "uterine milk" by the crypts of the cotyledon is, however, no longer accepted by modern investigators. Recent studies (Hammond, 1927 and others) have, indeed, made it nearly certain that the cotyledonary crypts have no maternal epithelium and are not secretory in the

true sense of the word ; this area too is quite free from the openings of any uterine glands. The fluid that is obtained by squeezing a cotyledon which has been separated from the foetal membranes is lymph (Hammond).

The fluid was first called "uterine milk" by W. Needham in 1667. According to J. Needham (1931) it was first analysed by Prevost and Morin in 1842, then by Schlossberger in 1855, and then again by Gamgee in 1864. Their results are incorporated in Table VI.

In addition to the substances included in Table VI, Bonnet (1882b), Kolster (1902, 1903, 1906), Jenkinson (1906, 1913), Hammond (1927) and others have been able to demonstrate, especially in the ungulates, the following constituents : leucocytes containing haemoglobin derivatives, fat globules, and occasional crystals—the "Uterinstäbchen" (Bonnet); tracts of glandular and surface epithelium (symplasma) set free by a process of "cellular secretion"; red blood corpuscles and their derivatives; connective tissue elements; enzymes (e.g. acid phosphatase), and hormones which are in solution and not recognisable by ordinary histological methods. Of these bodies and substances present in the uterine milk of ungulates, the protein component has been considered as being secreted by the surface and glandular epithelium, perhaps mingled with lymph transuded from the oedematous mucosa, whereas the fat droplets and the iron compounds contained therein have been regarded very generally as being liberated respectively from degenerating leucocytes and erythrocytes which had previously migrated, or somehow escaped into the uterine lumen (Bonnet, Kolster).

Besides the intracellular pigments, there are others sources of iron, though in widely varying amounts. In most placental Mammals a greater or less amount of maternal blood is in direct contact with the trophoblast, and the foetus usually utilises all such opportunities in varying degrees and in varying amounts to replenish its stores of iron. In some carnivores (e.g. dog, cat) blood extravasations occur regularly along the margins of the placental zone forming the so-called "green" and "brown" borders, and the evidence is all in favour of these characteristic extravasates providing the principal sources of iron for the developing foetus. In the ass, Strahl (1906a) has found blood in lesser amount, forming small effusions. In the sheep and cow its presence has often been noted by Tafani (1886), Bonnet (1899), Assheton (1906) Hammond (1927) and others (*see also* p. 201). And, finally, one should recall that in the sow and the mare, iron seems to be made available in the secretions of the uterine glands (p. 174) instead of being derived from leucocytes and erythrocytes which have undergone dissolution. In the sow it is found mainly in the recesses of the "areolae" and the uterine depressions which fit over them (Fig. 15. 26 b), whilst in the mare it accumulates in pockets on the chorion between cotyledons (i.e. where gland mouths open, Fig. 15. 28), as well as beneath the domes of trophoblast which overlie the endometrial cups (Fig. 15. 30).

Kolster (1902, 1903) has described a process by means of which cellular elements of the glands are added to the uterine milk. Small tracts of the epithelial wall become invaginated into the gland lumen, are cut off, degenerate and are ejected by the ducts giving rise to a glandular symplasma. It is not only the glandular epithelium which forms a symplasma. The surface epithelium may also give rise to a symplasma which may be of transient nutritional value to the developing embryo (*see* Fig. 15. 14 a).

TABLE VI
COMPARATIVE ANALYSES OF UTERINE MILK
(Quantities in gms. per cent. except where otherwise stated)

Constituent and/or Test.	Schloss- berger.	Gamgee.		Prevost & Morin.	Amoroso & Harvey (a).
	Cow.	Cow.	Ewe.	Cow.	Mare.
Water	88.07	87.91	88.30	86.84	79.64
Total Solids	(11.93)	(12.09)	(11.70)	(13.16)	20.36
Organic Solids	—	—	—	—	19.77
Inorganic Solids (ash)	0.71	0.37	0.45	—	0.59
Total Nitrogen	—	—	—	—	3.19
Protein Nitrogen	—	—	—	—	3.04
Protein (Protein N $\times$ 6.25 in last column).	9.60	10.56	9.90	11.03	18.00
Albumin	—	—	—	—	(b)
Globulin	—	—	—	—	None detected
Non Protein Nitrogen.	—	—	—	—	0.15
Fatty Matter	1.59	1.23	1.20	0.45	0.006
Reducing Substances (Hagedorn & Jensen)	—	—	—	—	0.5-0.6 mgm.%
Glucose (Phenylhydr- azine and Reducing Tests)	None	None	None	None	None detected
Fructose (Seliwanoff)	—	—	—	—	None detected
Glycogen	—	—	—	—	None detected
Biuret Test	—	—	—	—	+
Millon's (Tyrosine) . .	—	—	—	—	++
Sakaguchi (Arginine) . .	—	—	—	—	+++
Adamkiewicz (Tryptophan)	—	—	—	—	+++
Organic Sulphur (after sodium fusion)	—	—	—	—	++
Calcium	—	—	—	—	0.14
Chloride	—	—	—	—	0.2
Phosphate (as P)	—	—	—	—	0.2
Iron (after Sodium fusion—Ferron test)	—	—	—	—	—
Osmic Acid Test (Lipoid Double bond)	—	—	—	—	+
Salkowski's Reaction	—	—	—	—	+
Libermann-Burchardt Reaction (Cholesterol or other unsaturated Sterol)	—	—	—	—	+

Notes : (a) Unpublished data.

(b) The protein present is soluble in 22% sodium sulphate and is precipitated from its solution in 1% sodium chloride by large excess methanol or ethanol in typical "mucoid" like strands.

Traces of glycogen may be extracted from both the maternal and foetal parts of the cotyledons, but it is said to be too small in amount to be demonstrated histologically. It is also present in small amounts in the inter-cotyledonary areas—in the uterine epithelium both superficial and glandular in the cow, in the sub-epithelial connective tissue in the sheep, and in the uterine milk (Jenkinson, 1906). Large quantities of glycogen are stored in the *plaques amniotiques*,¹ localised masses of epithelial cells found on the internal surface of the amnion, and on the umbilical cord. In the bovine foetus the plaques reach their full development about the sixth month and then atrophy (Bernard, 1879).

While lying free in the uterine cavity, the uterine milk undergoes changes which consist largely in a disintegration of its cellular elements. The leucocytes degenerate and their cytoplasm, with the pigment granules, fat globules and "Stäbchen," is set free. The symplasma masses are also transformed into a mass of debris, and their constituents lie free in the *histotrophe*. The red blood corpuscles may be ingested almost unaltered by the trophoblast (Fig. 15. 14 c), or they may be first laked, and the haemoglobin may be absorbed as such, or undergo changes before absorption. According to Jenkinson, haemoglobin is broken up into an iron-containing and iron-free part. The former is carried away by the foetal blood vessels and stored in large quantities, principally in the foetal liver. The iron-free part is deposited in the cells as a pigment, occurring in such amount as to give, especially in the later stages of pregnancy, a deep brown colour to the foetal cotyledons. It collects at the apices of the villi, and its presence suggests that the cotyledons are actively engaged in excretion (Assheton, 1906). The histological changes in the red blood corpuscles absorbed by the trophoblast have been described by Jenkinson. They are engulfed by amoeboid processes of the cells, and gradually become paler in colour and irregular in outline; often they clump together. Gradually yellowish brown granules are deposited on the surface of the included cells, and this process continues till the whole is converted into a dark brown mass.

Bonnet was the first to show that the trophoblast in ruminants was actively phagocytic and absorbed the constituents of the uterine milk. After their absorption, the disintegration of the cellular constituents is completed in the trophoblast, and they are no longer recognisable as individual elements. Their products are transmitted to the foetal vessels, though they may first be elaborated in the trophoblast into a form or forms suitable for the use of the embryo in the development of the various organs.

Summing up, the cotyledonary and intercotyledonary parts of the placenta present differences both anatomically and physiologically. In the intercotyledonary region are the glands, and here only are found the gland-secretion and the secretion of the uterine epithelium. In the cotyledonary parts the glands are absent. Here the villi are formed, and they effect an attachment to the mucosa by the greater activity of the trophoblast. Assheton has suggested that this hyperactivity may be stimulated by the absence of glands and consequently of uterine milk in the cotyledons, the foetal ectoderm attacking the mucous membrane more vigorously in order to obtain food. The blood effusions are also cotyledonary and iron reactions are obtainable in the adjacent trophoblast, and not at other

¹ They have been variously known as *amniotic pustules* (Hammond, 1927), *amniotic villi* (Grosser, 1927; Wislocki, 1941), *amniotic caruncles* (Heuser and Wislocki, 1935).

places. Finally it is probable that the exchange of oxygen and carbon dioxide is carried out principally in the cotyledons. Here the maternal capillaries come close to the surface of the mucosal trabeculae, some of them even indenting the lining membrane of the crypts. The chorionic epithelium is likewise deformed by the allantoic capillaries which, in some forms, pursue an intraepithelial course, so that a very close relationship is established between the two circulations. In the intercotyledonary regions, on the other hand, the foetal vessels are related to the orifices of the glands, and appear to be concerned principally with the absorption of the less diffusible substances transmitted or secreted by the surface and glandular epithelium.

IX. PLACENTA ENDOTHELIOCHORIALIS

In this type of placenta the structural arrangements by which the trophoblast is able to secure nourishment for the embryo from the wall of the uterus, bear a certain resemblance to the conditions found in the syndesmochorial variety just described, but, as we shall see more fully in the next section, differ widely from those encountered in the haemochorial and haemoendothelial types.

In all cases where an endotheliochorial placental relationship is developed, implantation is central¹ and the attachment of the blastocyst to the uterine wall is accomplished over a relatively wide equatorial zone, comprising, in some cases, a third or even more of the surface of the chorion. In the neighbourhood of the attachment the mucosa degenerates, and the trophoblast comes to enclose the endothelial walls of the capillary vessels in which the blood of the mother circulates. At the same time, the uterine tissues interlock so closely with the foetal villi that the two cannot be separated without injury, and at birth the placenta tears away as a unit, leaving behind a basal, deeper, regenerative zone of less modified mucosa. While, therefore, endotheliochorial and haemochorial placentae have this much in common that the uterine epithelium disappears, they differ wholly from each other in that, in the latter, the maternal blood circulates not in blood vessels enclosed by the trophoblast but in lacunae, excavated in that tissue. On the other hand, it must be emphasised that the maternal vessels which become incorporated in the placental labyrinth of the endotheliochorial variety still retain a definite, but extremely delicate perivascular coat of argyrophil fibrils. Indeed, as Wislocki and Dempsey (1946a) have remarked, it is doubtful whether such a placental relationship could exist without some sort of matrix to reinforce the endothelial wall. Consequently, the endotheliochorial labyrinth, in that it consists of bands of trophoblast and of delicate strands of argyrophil fibrils surrounding intact maternal blood vessels, does indeed resemble the syndesmochorial, ungulate type, from which it may have been conceivably derived (Jenkinson, Grosser, Hill and others). There is, however, no universal agreement in this matter, and those who like Wislocki (1929), maintain that some sort of invasion by the trophoblast is more primitive, interpret these findings in the opposite way.

¹ *Nyctalus noctula* appears to be an exception, though according to Wimsatt (1944), Mossman (1937) has misconstrued the statements of van der Stricht (1899, 1911) and assumed incorrectly that implantation in this bat is interstitial.

An endotheliochorial placental relationship is characteristic of the Carnivora and the Megachiroptera (Grosser, 1927). A placenta of this kind is also found in at least three of the sixteen families of Microchiroptera (Hamlett, 1934, 1935b ; Mossman, 1937) ; in *Bradypus griseus* and *Choloepus hoffmanni* (Xenarthra), the tri-dactyl and di-dactyl Sloths (Wislocki, 1926, 1927, 1928a, 1928b ; Heuser and Wislocki, 1935), and, amongst the Insectivora in several species of the three families *Talpidae*, *Soricidae* and *Tupaiidae* (Grosser, 1927, 1936 ; Mossman, 1937, 1939b ; and Wimsatt & Wislocki, 1947). According to de Lange (1933), the placenta of the aardvark (*Orycteropus*) is said to be of the same invasive and deciduate type, but this is denied by Mossman (1937). Huxley (1869) appears to have been the first to suggest that the placenta of the Cape anteater was zonary and deciduate.

Our present knowledge of the histological structure and development of the endotheliochorial placenta is derived principally from the condition as it is observed in Carnivora. The forms most frequently investigated have been the dog, the cat and the ferret. Years ago, von Baer (1828) and Bischoff (1865a, b), studied the process exhaustively in the dog and other carnivores, whilst Duval's copiously illustrated studies which appeared between the years 1893 and 1895, as well as those of Schultze (1896) and Strahl (1906a) give authoritative information concerning it in both the dog and the cat. Other studies are those of Heinrichs (dog, 1889 ; cat, 1891), Bonnet (dog, 1897a, 1901, 1902, 1903a), Schoenfeld (dog, 1903), Robinson (leopard, cat, ferret, 1904), Strahl and Ballmann (ferret, 1915), Subba Rau (*Zorilla* and brown bear, 1925), Grosser (dog and cat, 1927, 1936), de Lange (dog and cat, 1933), Janssen (cat, 1933) ; while the first extensive histochemical characterisation of the placenta of any carnivore was the careful investigations of Wislocki and Dempsey (1946a) of the cat's placenta. The following account refers particularly to the cat and dog.

The Foetal Membranes and Placenta of the Cat and of the Dog

Gestation, litter size, ovulation and tubal transit.

In the cat sexual maturity is reached at about 9 to 15 months, while in the dog puberty is reached at 6 to 8 months (Gerlinger, 1925). The gestation period in the cat is on an average sixty days, and in the dog fifty-eight to sixty-three days. Another average given for the cat is sixty-three days (Gros, 1936). In the present study the mean litter size of the cat was 3.68 ± 0.102 which is slightly smaller than the 3.88 recorded by Hall and Pierce (1934) ; there is no precise information for the litter size of the dog (*see*, however, Asdell, 1946).

In both, the ovum takes a comparatively long time to traverse the oviduct. In the cat the eggs enter the uterus some time during the fifth day after ovulation ; this is also the view of Gros. Other authors, however, place the time as late as the sixth or even the seventh day (Hill and Tribe, 1925 ; Manwell and Wickens, 1928). But, unlike the cat, which ovulates only after coitus (26 to 30 hours) or some comparable stimulus (Greulich, 1934 ; Amoroso, 1940 and others), ovulation in the bitch is spontaneous and usually takes place 1 to 3 days after first acceptance (Evans and Cole, 1931 ; Griffiths and Amoroso, 1939), the eggs entering the uterus six to eight days thereafter.

Preimplantation changes in the uterus.

As the several phases of endometrial behaviour have been described in detail and carefully illustrated by Courrier and Gros (1932a ; 1932b ; 1933), Gros 1933 ; 1936), and Dawson and Kosters (1944) in the cat, and by Marshall and Jolly (1906), Keller (1909) and Evans and Cole (1931) in the dog, only a brief outline of the more general features will be given here. The glandular transformations during progestation and pregnancy are very much alike in the two species.

In the first week following ovulation the mucosa becomes increasingly folded and there is a progressive development of the uterine glands and crypts accompanied by the formation of a well differentiated, subepithelial, cellular layer. The enlargement of the uterine glands results both from the hypertrophy of the epithelium, and to a lesser extent from dilation of the gland lumina. Mitoses are abundant throughout. This cellular proliferation is accompanied by vacuolization of the individual cells, which are pervaded with minute fat droplets and intracellular deposits of glycogen (Bonnet, 1897b ; Melissinos, 1906 ; Dawson and Kosters, 1944). Moreover, Wislocki and Dempsey (1946a) report the occurrence in the surface and glandular epithelium, in both species, of deposits of calcium, an abundance of stainable alkaline and acid phosphatase and a pronounced cytoplasmic basophilia which also involves the cells of the subepithelial connective tissue layer. As a result of the glandular hypertrophy the stroma of the endometrium is progressively thinned out and the typical radial disposition of the glands about the uterine lumen becomes obscured. The crypts provide an increase of superficies and of secreting epithelium and are later concerned in the attachment of the ovum.

Important changes also occur in the blood vessels. As in other mammals, the endometrium becomes increasingly hyperaemic during this period and miliary haemorrhages may sometimes occur from the rupture of some of the superficial capillaries. But at this stage there is no break in the continuity of the surface epithelium.

During the second week of the preimplantation period, the endometrial epithelium continues to hypertrophy and the mucosal folds becoming more prominent project into the uterine cavity, giving it a fringed or scalloped appearance. The uterine glands now open into the bases of these crypt-like spaces by narrow necks. The glands, which are moderately tortuous, widen quickly into "chambers," and tracts of their proliferated epithelium are invaginated and often obliterate the lumen. The widening of the glands together with the restriction of interglandular connective tissue make the deeper layer spongy in contrast to the superficial *compact zone* (Fig. 15. 46). It is within the *spongy zone* that the major portion of the glands lie. The capillaries of the adjacent stroma increase, become dilated and sinus-like and form practically the whole of the subepithelial layer. Immediately below it lies the layer of glandular ducts which are obliterated by debris resulting from the degeneration of the proliferated epithelial cells. Between it and the spongy layer is the compact layer, also formed from the subepithelial layer and characterised by marked crowding of its cells. In it the necks of the glands are narrow and the connective tissue is more abundant. Below the spongy zone lies the muscularis. However, in the dog (Fig. 15. 46) there is a thin connective tissue layer through which prolongations of the glands of the spongy

layer pass. This is followed by a deeper layer of unaltered ends of the glands adjacent to the muscular wall. Most of the glands are destined eventually to be destroyed by the invasive trophoblast, but even near term glandular remnants may be found in the subplacental region.

The extensive histological alterations of the surface and glandular epithelium during the pre-implantation period reflect the initiation of secretory activity. The uterine cavity contains a moderate number of leucocytes and these may also be found between the epithelial cells. The histotrophe at this stage differs from

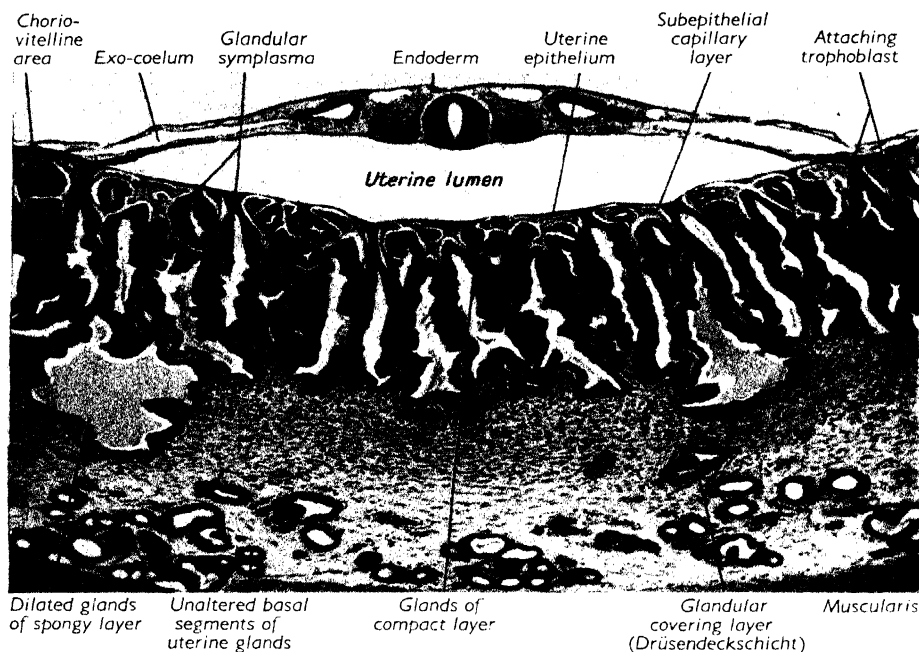


Fig. 15. 46—Early stage in the attachment of the trophoblast to the wall of the uterus in a bitch 17 days pregnant. Note that the embryonic pole of the blastocyst has not as yet united with the uterine wall. $\times 50$.

that of Ungulates. The glandular secretion is less fluid, perhaps because the lymph transudate is less abundant (Kolster, 1906), and doubtless also, because there is a more extensive degradation of the glandular epithelium. It surrounds the ovum to form the *prochorion* or "Gallertschicht," and is according to Bonnet (1897b) absorbed by the trophoblast.

Some light has been thrown upon the problem of the pre-implantation transformation of the endometrium in Carnivora, by studies on non-gravid cornua and of pseudopregnant uteri (Keller, 1909; Hammond and Marshall, 1930; Evans and Cole, 1931; Courier and Gros, 1933 and others). When pseudopregnancy is induced in the cat, either by a sterile mating (Gros, 1935; Liche, 1939), by mechanical stimulation of the cervix (Dawson and Kosters, 1944), or by hormonal treatment (Foster and Hisaw, 1935; Rowlands and McPhail,

1936; van Dyke and Li, 1938 and others), the uterine changes which follow parallel, with some slight retardation, those found at the time of implantation of fertilised ova. The duration of this pseudopregnancy is, however, very variable. In the cat, for example, Gros (1935), finds that after sterile copulation the active phase of pseudopregnancy lasts about 23 days after ovulation and the regressive phase about 10 days. Foster and Hisaw, on the other hand, report an active phase of 30 days in a pseudopregnancy induced by the injection of FSH and LH while, according to van Dyke and Li, pseudopregnancy is no longer present 20 days after hormonal induction of ovulation. In the bitch Evans and Cole state that glandular regression appears to begin about the twentieth day of metoestrus, with the uterus usually in repose on the ninetieth metoestrous day. Probably as Asdell (1946) states it is too gradual to set any definite limits. There is a considerable literature on this subject which may be approached through the papers cited above (*see also* Chapter 19).

Spacing and orientation of the blastocysts.

Very shortly after entering the uterus, the blastocysts become spaced more or less evenly throughout its length. As a rule the embryo lies antimesometrially with the embryonic axis almost transverse to the long axis of the uterine cornu (Fig. 15.47). The spacing and orientation take place prior to permanent contact being established with the uterine mucous membrane, and by the

thirteenth day the location of the implantation sites are recognisable, in the cat, as appreciable uterine swellings measuring from 9 to 10 mm. in diameter. In the dog the locular enlargements are first noticeable on the fifteenth day.

A peculiarity of the location of the uterine swellings in these species, and one which results from the internal migration of the eggs,¹ is the fact that there is a very marked tendency to equality of distribution of embryos between the two uterine cornua (Markee and Hinsey, 1933). That this distribution is not fortuitous can also be shown by comparing the observed distribution of implanted embryos and of old placental sites in parous animals with the expected random distribution as given for the cat in Table VII.

¹ In the present study migration of ova in the cat has been observed in 20% of all post-implantation stages examined. Migration has also been recorded in a case of congenital absence of the left ovary and also following unilateral oöphorectomy. (See, however, Chapter 14.)

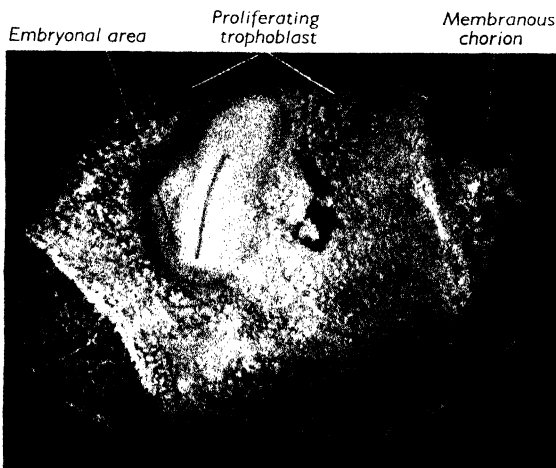


Fig. 15.47—Blastocyst of the cat removed from the uterus on the 13th day of pregnancy, showing the zone of proliferating trophoblast and the orientation of the embryonic disc. Note the membranous poles of the chorion. $\times 7\frac{1}{2}$.

TABLE VII

Difference of number of embryos in the two uterine cornua.	Observed.			Divergence of sum of the ex- pected from observed values.	χ^2/m
	Expected.	Embryos.	Placental sites.		
6	0.3	0	0	- 0.3	3.7
5	3.4	0	0	- 3.4	
4	13.3	0	0	- 13.3	
3	35.0	4	3	- 28.0	22.4
2	66.7	15	12	- 39.7	23.63
1	94.6	64	62	+ 31.4	10.42
0	53.7	54	53	+ 53.3	52.9
	267.	137	130	0.0	$\chi^2 = 126.35$

Implantation.

In the Carnivora—and the cat and dog are no exceptions—implantation is of the *central* type, in which the blastocyst rests in the cavity of the uterus, attains a large size, and comes in contact with the wall over its whole circumference. But as the vesicles mature, the trophoblast at the poles lags behind the invasion of the trophoblast in the central zone, and by the time implantation is completed, the definitive placental area may be reduced to a girdle-like band about the middle of the chorionic sac, a single semicircular patch, or one or two disc-shaped areas (cf. Figs. 15. 11 c and 12).

In the cat the time when the ovum becomes implanted has been placed as early as the eleventh day and as late as the nineteenth day after copulation (Manwell and Wickens, 1928; Duval, 1894). However, the extensive observations of Courrier and Gros (1932a, 1932b, 1933) and Gros (1936), indicate that attachment occurs between the thirteenth and fourteenth days, and personal findings are in agreement with these observations. In the greyhound bitch the blastocysts implant $16\frac{1}{2}$ to 17 days after ovulation (Griffiths and Amoroso, 1939), that is about 18 to 20 days after first acceptance, as was first suggested by Duval (1893). In procuring attachment to the uterus, the epithelium is destroyed leaving exposed a superficial layer of stroma. In doing so the endometrium is invaded by solid, tongue-like masses of trophoblast (Figs. 15. 46 and 48). The latter occurs at first mainly as cytotrophoblast contiguous to the foetal mesoderm but, later, partly also as syncytial trophoblast constituting the outlying and more invasive layer. Many of these solid trophoblastic villi project into glands and crypts and block their openings; others are said to obtain attachment at parts of the surface where there are no gland openings or crypts, and penetrate into the substance of the mucosa (Strahl, 1890a; 1890b; Duval, 1893). The stroma appears to react in a characteristic way to the presence of the trophoblast by proliferation and enlargement of its cells (decidual reaction) and by dilation of the large number of small maternal blood vessels. Before the disappearance of the epithelium, the cells lose their outline and form a homogeneous mass of protoplasm with fragmented nuclei.

This degenerated tissue ought not, as Bonnet (1903b) emphasised, to be known as *syncytium*, which represents an active protoplasmic condition. The name which he suggested, *symplasma*, is very convenient and is used here. It is not only the surface epithelium which forms a symplasma. The glandular epithelium, the connective tissue cells, and extravasated blood may also give rise to a symplasma which may be designated *glandular*, *connective tissue*, and *haematogenous* respec-

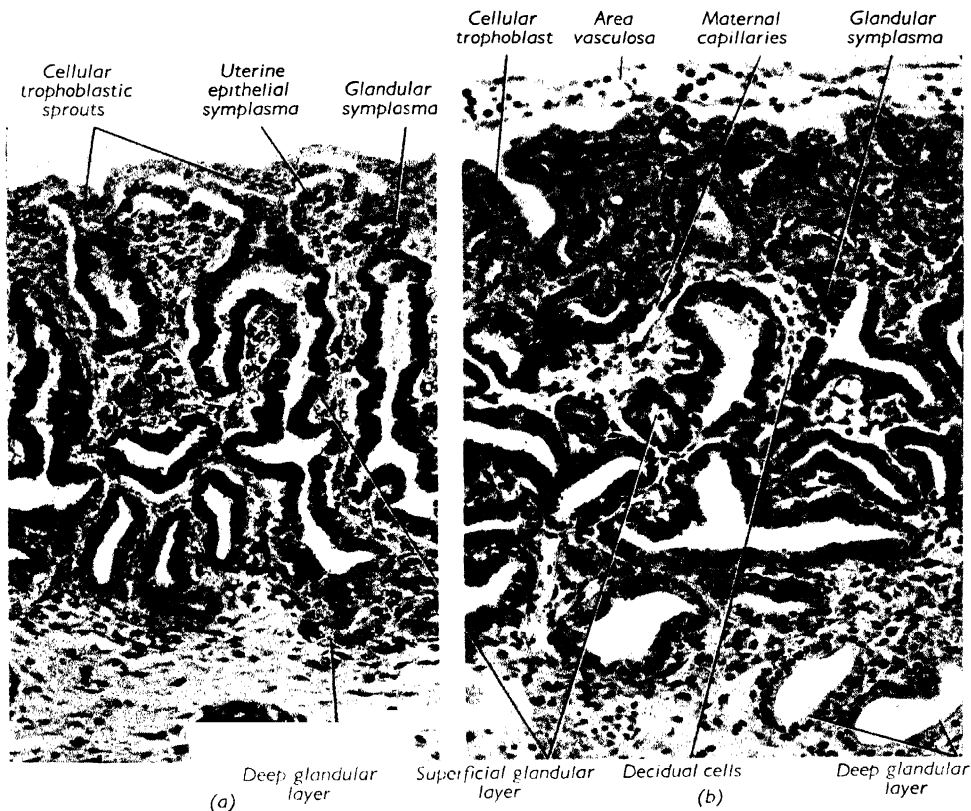


Fig. 15. 48—(a) Early stage in the attachment of the trophoblast to the wall of the uterus in a cat 13½ days pregnant. $\times 133$. (b) A portion of the chorio-vitelline placenta of a cat slightly earlier than that represented in Fig. 15. 10. $\times 133$.

tively. All are formed to a large extent in the placenta of Carnivora, and their resemblance to the trophoblastic syncytium has led to much confusion.

In its advance into the maternal tissues the trophoblast destroys not only the lining and glandular epithelium, but much—though not all—of the subjacent decidua as well. On the other hand, the maternal capillaries remain intact, their endothelial walls, supported by a reticular stroma with occasional stromal cells, becoming completely surrounded by trophoblast. This mode of invasion of the endometrium by the trophoblast results, therefore, in the establishment at a very early stage of an endotheliochorial placental relationship in which the

blood circulation in the foetal capillaries (area vasculosa) is separated from that in the maternal blood channels by the maternal endothelium, supported by wisps of argyrophil fibres, a thin layer of the cellular trophoblast, an irregular layer of mesenchymal cells (which is sometimes deficient locally) and the delicate endothelial wall of the foetal blood vessels. In the course of the foregoing changes, the embryonic segment of the blastocyst remains exposed to the uterine lumen for a relatively long time, and does not unite with the antimesometrial wall of the uterus until the chorio-amniotic folds have fused, thus completing the circular equatorial zone of attachment of each blastocyst with the mucosa. (Fig. 15. 46.)

By the end of the implantation period two different placental regions of the trophoblast may be recognised (Fig. 15. 10). These are :—(1) the trophoblast of the vascular trilaminar omphalopleur (chorio-vitelline placenta) which is applied to the mesometrial wall of the implantation chamber and, (2) the non-vascular extra-embryonic somatopleur (chorionic placenta) constituting the antimesometrial wall of the locular enlargement. Both these regions contribute to the genesis of the chorio-allantoic placenta, the transformation being brought about when vascular allantoic mesoderm reaches and makes contact with the non-vascular chorionic area. (*See below.*)

The yolk-sac and yolk-sac placentation.

In both the cat and the dog, the yolk-sac is completed while attachment of the ovum with the maternal tissues is being effected, and in association with the overlying trophoblast enters into varying physiological relationships with the endometrium. The yolk-sac cavity is at first very large, its upper wall indented by the embryo results in the so-called "incomplete inversion" of the sac. But in the later stages, when the sac has been separated off as an independent vesicle, it becomes much reduced. Three stages in its development may be recognised. These are :—(1) the non-vascular bilaminar omphalopleur; (2) the non-vascular trilaminar omphalopleur; and (3) the vascular trilaminar omphalopleur or chorio-vitelline placenta.

In the first of these, the endoderm of the yolk-sac is in contact throughout the extra-embryonic region with the trophoblast and forms with the latter the bilaminar omphalopleur, the foetal component of the yolk-sac placenta during its first stage of development. The bilaminar omphalopleur is non-vascular and is completed in both species before implantation. In the second stage the area of the bilaminar omphalopleur diminishes progressively as the mesoderm advances between the endoderm and the trophoblast transforming the bilaminar into the transitory trilaminar omphalopleur, also non-vascular. Finally, in the third and last stage this non-vascular trilaminar structure is invaded by the vitelline vessels of the area vasculosa and transformed into the vascular trilaminar omphalopleur which is the foetal component of the chorio-vitelline placenta. As in the other carnivores there is no sinus terminalis.

The chorio-vitelline placenta is established in the early somite stages of the embryo and is the principal placental organ during the period of nidation. Reference again to Fig. 15. 10 will show the essential points in its development and morphology in the cat. It disappears eventually between the twenty-first and the twenty-fourth day when the extension of the exo-coelom into the area vasculosa separates it into the vascular splanchnopleur and the non-vascular somatopleur.

The process of separation of the vascular layer is not completed until sometime after the vascular allantoic mesoderm has reached and made contact with the area of chorionic attachment (which is forming antimesometrially), to initiate the chorio-allantoic circulation. However, this disruption of the chorio-vitelline attachment cannot mean the end of the functional importance of the yolk-sac, for it persists in both species till birth as a wrinkled, richly vascularised sac which is attached by its extremities to the chorionic membrane. Moreover, studies now in progress indicate that in addition to its haemopoietic functions it accumulates a fluid of high nutritional value for the foetus.

In its finer morphology the chorio-vitelline placenta is a simple structure, consisting, for the most part, of solid trophoblastic villi which have engaged the endometrium and have penetrated the mucosa to variable distances (Fig. 15. 48 b). The uterine epithelium has disappeared and glandular symplasma blocks the eroded mouths of the crypts. In its advance the trophoblast thickens but remains cellular and does not become invested with a syncytial mantle until the trophoblastic villi have reached an appreciable thickness. The invasion of these villi by mesoderm is extremely feeble and the mesodermal cores are completely devoid of a foetal circulation (Fig. 15. 49). Thus in the chorio-vitelline placental area, the only region of intimate association of the foetal and

maternal circulation is in the neighbourhood of the confluent maternal sinuses which are located at the base of the villi (i.e. on the placental face) and which are separated from the vitelline capillaries by an extremely thin layer of cellular trophoblast (Placenta endotheliochorialis). The invading trophoblast dips into the masses of glandular detritus and apparently absorbs them for they eventually disappear completely, and doubtless provide a source of nutrition for the developing embryo. Histochemically the endodermal cells of the chorio-vitelline placenta are characterised by heavy deposits of fat and exhibit faint traces of glycogen during



Fig. 15. 49.—Detail of chorio-vitelline placenta of a cat about 14½ days pregnant, showing three solid villi covered by trophoblast which is partly cellular and partly syncytial. Note the feeble invasion of mesoderm. S. 340.

the middle of pregnancy, whereas alkaline phosphatase appears and gradually increases during the last two weeks of the gestation period.

Concerning the mechanism which causes the degeneration of the uterine epithelium, there is still considerable doubt. Van den Broek (1904) suggests that the general oedema of the uterine mucosa may lead, as elsewhere, to its malnutrition, and thus it may fall an easy prey to the trophoblast. Others maintain that the degeneration is brought about by a trophoblastic influence, perhaps of the nature of an enzyme action (*see* p. 285). However, the occurrence of local deposits of glycogen in the surface epithelium and in the necks of the uterine glands at the implantation sites, affords some confirmation of the view that the degenerative changes may also result from the reduction in circulatory efficiency that characterises glycogen deposition (Dempsey and Wislocki, 1944). The result is a transformation of all the elements to a symplasma.

Chorionic placentaion.

Reference to Fig. 15. 10 will show that prior to its vascularisation by allantoic vessels, there is an appreciable area of non-vascular extra-embryonic somatopleur which is intimately fused with the endometrium in the embryonic hemisphere of the implantation site. This region of chorionic attachment comes into existence mainly as a result of the devascularisation of the chorio-vitelline placenta, but partly also as a result of the fusion of the chorio-amniotic folds. It forms a transient, supplementary structure for the interchange of materials between the maternal blood and the exo-coelom, and into which they have come by passing through the walls of the mucosal capillaries and the ectodermal and mesodermal laminae of the chorion. The chorionic placenta of the cat described here has received relatively little attention in the past. But it is apparent that until the chorio-allantoic circulation is established, and during the period of functional decline of the chorio-vitelline placenta, it must play a part in the transmission of substances of value to the foetus.

Chorio-allantoic placentaion.

The chorio-allantoic placenta is fundamentally alike in the cat and dog. It is a zonary and typically deciduate placenta with the trophoblast coming in contact with the intact walls of the maternal capillaries. As earlier indicated, however, the separation membrane between the two blood-streams probably does not thin out to the point where the interstitial matrix surrounding the maternal vessels disappears completely ; if this condition occurs it is only in a very few places and in very late stages.

Early in development, the blastocysts are spherical and the trophoblast is equally thick and well developed on the whole surface of the sphere, but as pregnancy advances the vesicles elongate rapidly, acquiring completely membranous poles with the definitive placental area reduced to a relatively narrow equatorial girdle (*see* Figs. 15. 11 c, 12, 47). In the cat, at full term it measures about 45 to 50 mm. across its greatest width and 5 to 7 mm. in thickness at its centre whence it tapers towards its margins ; the outer margin of the band is occupied by the marginal haematoma (Fig. 15. 12). The weight of the placenta varies from about 17 to 20 gr., which is about ten per cent. of the weight of the whole conceptus. The umbilical cord is usually inserted centrally along the mesometrial border of

the placental girdle, and the vitelline vessels (pictured by Needham as early as 1667) pass directly to the walls of the persistent yolk-sac.

The allantois and amnion.—The allantois is large and, as in the mare, completely encloses the embryo enveloped in the amnion, but in such a way that the yolk-sac is compressed against the mesometrial wall of the gestation sac, where it remains until term as a collapsed but well-vascularised sac (Fig. 15. 50). The allantoic portion of the cord is exceedingly short.

The wall of the amnion is thin and is separated from the overlying allantois by the extra-embryonic mesoderm. There is generally a large proamnion—that region of the amnion below the head of the embryo from which mesoderm is absent—but this does not persist. Amniotic pustules such as occur on the amnion of the sheep and cow are altogether absent.

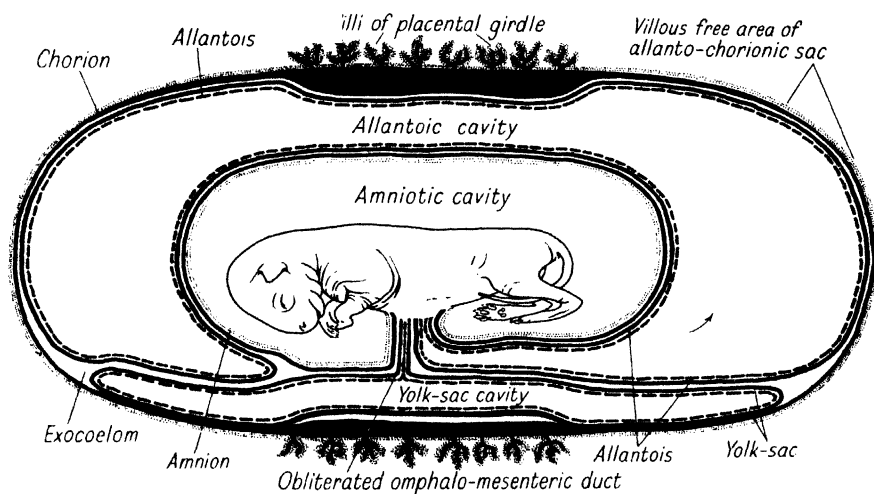


Fig. 15. 50—Diagram of the allanto-chorionic sac of the cat before complete regression of the chorio-vitelline placenta. Note the two parts of the umbilical cord.

Measurements of the volume of the amniotic and allantoic fluids in the cat are given by Wislocki (1935a). The volume of amniotic fluid rises slowly in a linear way until the foetuses are about 90 mm. long (10–15 ml.), after which there is a slight fall followed by a rise just before term. At term the mean weight of the amniotic liquid is about 16.5 gm. that is, about 8.75 per cent. of the weight of the whole conceptus. The allantoic fluid behaves differently from the amniotic fluid. The initial rise is more rapid and the peak reached at mid-term is higher (20 ml.), while towards the end of gestation it declines very perceptibly and at a mean weight of 6.5 gm. comprises about 4 per cent. of the weight of the locular enlargement (Author). For further details see Zietzschmann (1924).

Outline of development of the allantoic placenta.—Attention has already been drawn to the fact that the allantoic placenta is initiated at the time of implantation, but its characteristics are not fully developed until the second half of pregnancy. The main changes are effected by proliferation of foetal tissues and may be stated

as follows : At the end of the third week of pregnancy (on the eighteenth day in the cat and the twentieth day in the dog) vascular allantoic mesoderm reaches and makes contact over a broad zone with the chorion of the antimesometrial hemisphere. Apparently as a result of this contact and the blood supply it brings, the trophoblast begins to proliferate rapidly. This tissue contains many mitotic figures and now appears generally more virile than the trophoblast of the abembryonic pole (chorio-vitelline area). The primary villi penetrate into the deeper tissues of the mucosa by gradually absorbing the symplasma, both glandular and connective tissue, but sparing the maternal vessels. In their incursions into the endometrium, the trophoblastic villi soon acquire a vascular mesenchymal core by the ingrowth of allantoic mesoderm, and branch to form secondary and tertiary villi.¹ At the same time the trophoblast on the sides of the villi becomes more obviously syncytial, but retains its cellular character at the tips. Thus is formed the *angioplasmode* of Duval—a continuous layer of foetal vascular villi, clad with syncytium, penetrating everywhere into the capillary layer, and leading to a disappearance of all the maternal tissues except the vessels.²

When first formed, the syncytial trophoblast is characterised by an intense cytoplasmic basophilia and it thickens rapidly by increment from the cellular trophoblast, enclosing maternal capillaries and decidual cells in this process. A distinctive distribution of phosphatase also characterises this invasive layer of the trophoblast, the reaction becoming more vivid as term is approached, whereas the basophilia decreases. Expressed chemically, this occurrence of cytoplasmic basophilia suggests the presence of ribonucleo-protein which probably plays a rôle in protein synthesis during a period of active endometrial growth and secretion (Wislocki and Dempsey, 1946a), whereas the presence of phosphatases indicates the existence of metabolic reactions involving dephosphorylation.

As mentioned above the apex of each villus is capped by cellular trophoblast. At the mouths of the glandular crypts the convexity of the outer surface of the trophoblastic arcades is matched by a concavity of the inner surface and it is into these broad villi that the vascular allantoic mesoderm grows. Moreover, as it is from these structural units that the placenta is built up, many of its features, such as the direction of the maternal and foetal blood through it, are dependent on this mode of development. By the cytotrophoblastic plates at the tips, the layer of villi rests on the sheet of glandular detritus and the compact layer, which in turn form a symplasma and undergo absorption. Thus the foetal structures (villi) reach the spongy layer, in which the glandular crypts have expanded to form large cavities separated by vascular partitions carrying maternal vessels, the interglandular septa. Gradually the roof of this layer is also absorbed by the trophoblast, and the ectodermal arcades at the tips of the villi gain a permanent attachment to the interglandular septa. At the same time, by the further branching and penetration of the foetal mesoderm in the *angioplasmode*, the tissue is broken up into a series of labyrinthine lamellae, which consist of a network of maternal vessels clothed on each side by syncytial trophoblast. The meshes of the network are penetrated by the vessels of the villi. In this way the placental *labyrinth* is established, which by the end of the fourth week has acquired the characteristics

¹ It is very probable that there is also a local production of mesenchyme by cytomorphosis of the cellular trophoblast.

² In the cat a few decidual giant cells are present.

of the ripe placenta (Fig. 15. 51). In it the maternal and the foetal blood are separated by the endothelium of the uterine capillaries, the giant cells (absent in the dog), the trophoblastic covering of the villi, a thin mesenchymal layer and foetal capillary walls.

From now onwards throughout the gestation period the changes in the placenta are purely of the nature of an elaboration of pre-existing structure.



Fig. 15. 51—Section through the placenta and uterine wall of the cat at the 25th day of pregnancy. $\times 120$.

Detailed histology of the chorio-allantoic placenta.

In vertical sections of the placental zone of stages similar to and more advanced than that represented by Fig. 15. 51, the following parts are recognisable :—

- (1) The region of the placental labyrinth.
- (2) The transition, penetration or junctional zone.
- (3) The deep glandular or spongy zone.
- (4) The central and marginal haematomas.



Fig. 15. 52.—Section through the chorio-allantoic placenta and uterine wall of the cat near term. $\times 24$.

The placental labyrinth.—In the cat, this zone consists of more or less vertically disposed lamellae enclosing the maternal capillaries. It is comparatively thick and comprises two-thirds or more of the sectioned surface of the placenta (Figs. 15. 51

and 52). Between the lamellae are strands of allanto-chorionic mesenchyme in which are situated the thin-walled foetal capillaries. The latter vary considerably in size, being smallest where they underlie the trophoblast. The maternal capillaries, on the other hand, are more or less of uniform size and their lining cells are somewhat hypertrophied. The trophoblast covering the surface of a lamella consists of a superficial, lightly staining, cellular layer closest to the foetal stroma and a deep, basophilic, syncytial layer closest to the maternal tissues (Fig. 15. 53). Between neighbouring syncytial layers are situated the thick-walled maternal capillaries, and also large pale staining, decidual cells sometimes with more than one nucleus (the "cellules centrales" or "cellules géantes" of Duval). These cells occasionally exhibit mitotic activity. Surrounding the giant cells and maternal capillaries, and intervening between them and the syncytial trophoblast is a thin, but distinct layer of interstitial argyrophilic material which exhibits a positive reaction for phosphatase during a good part of gestation. These regions in the cat placenta present the most intimate approximation of the circulating maternal and foetal blood and the definitive placental membrane is thus seen to be comprised of the thin foetal endothelium, an irregular mesenchymal lamina, the cellular trophoblast, the syncytial trophoblast, a fenestrated layer of argyrophil fibrils¹ and the thickened endothelium of the maternal capillaries. It must be pointed out, however, that throughout approximately the last half of pregnancy, the layer of cellular trophoblast is destined gradually to lose its continuity until, at term, it has all but disappeared

and survives only at the base of the placenta as a vascularised layer which occludes the mouths of the glandular crypts (see Fig. 15. 56). Moreover, at many points where the cytotrophoblast is deficient, the foetal capillaries come to lie so near the surface of the lamellae that only the thinnest laminae of syncytial trophoblast separates them from the maternal tissues, thus reducing still further the thickness of the placental membrane. These facts are of the utmost importance; for as Pohl and Flexner (1941) have shown, this progressive attenuation of the limiting membrane is accompanied by an increase in the unit transfer rate of radioactive sodium across the placenta as term is approached,



Fig. 15. 53 — Details of the placental labyrinth of the cat at about the 26th day of pregnancy. $\times 430$.

¹ In the cat's placenta at term, only occasional reticular fibres surround the giant cells.
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suggesting "that changes in placental transfer rates are in considerable part due to changes in placental permeability."

Various opinions have been expressed regarding the origin of the giant cells. They were generally looked upon by earlier workers (Duval, 1895; Grosser, 1927) as of trophoblastic derivation, but in view of personal findings and of the

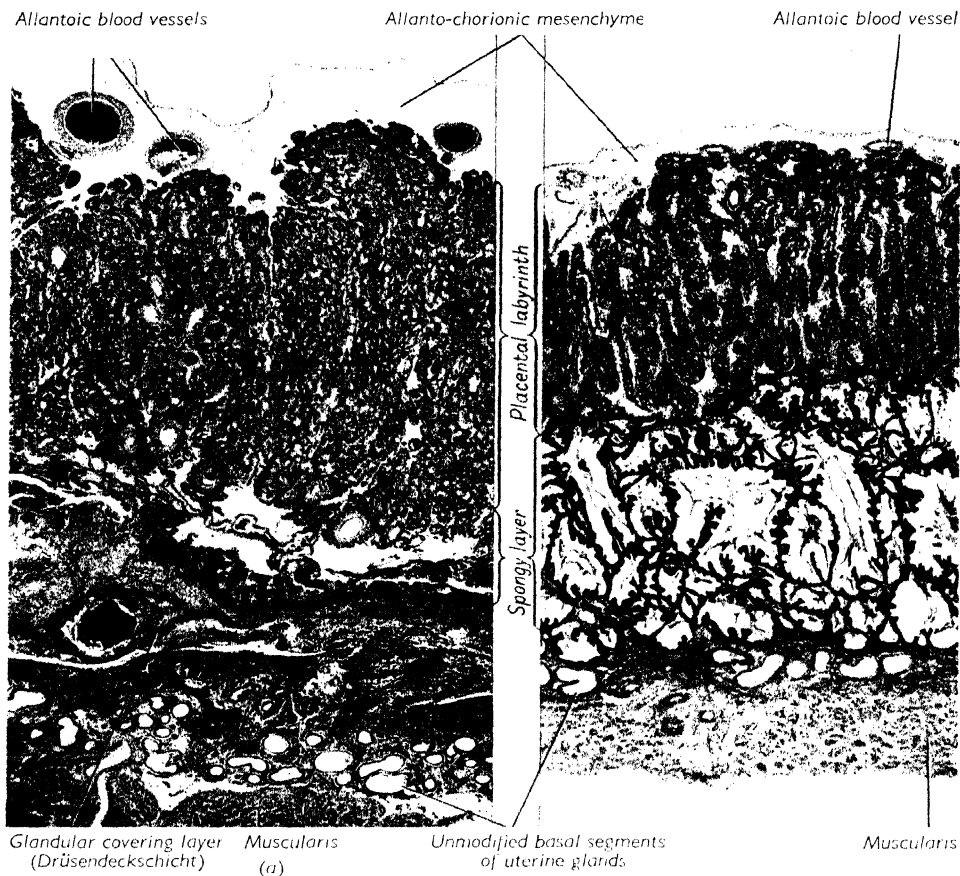


Fig. 15. 54—(a) Section through the placenta and uterine wall of the dog near term. *Fig. 19.*
(b) The labyrinth and spongy zone of the placenta of the sea lion (*Zalophus californianus*) for comparison with that of the dog. *Fig. 22.*

results obtained by Wislocki and Dempsey (1946a), the most probable interpretation is that they are decidual cells which have become incorporated along with the maternal vessels into the placental lamellae. Wislocki and Dempsey list a number of histochemical tests by which they may be distinguished from both the cellular and syncytial trophoblast. Moreover, the presence of argyrophilic fibrils surrounding the giant cells and maternal capillaries and intervening between them and the trophoblastic syncytium, they regard as a matter of the greatest importance in supplying the main proof of a decidual origin of these cells.

In respect of the syncytium, the most diverse accounts have been given of its origin and later history in the placenta of the Carnivora. Most of the earlier writers, relying mainly on staining reactions, regarded it as originating from the uterine epithelium (Strahl, 1890a, b) or as being a product of the fusion of maternal and foetal epithelia, the so-called foetal-maternal "diploplasma" in de Lange's terminology (Bonnet, 1907; Schoenfeld, 1903). Others (e.g. Heinricius, 1889) have accepted the probability that the epithelium disappeared and that the syncytium was formed by the uterine connective tissue. These observations, however, stand alone. Later writers, notably Grosser (1927), and Wislocki and Dempsey have hesitated to accept these conclusions, and it is now generally recognised that the syncytium is trophoblastic. The only doubts that remain relate to the mechanism of its growth. However, when this question was studied with the colchicine technique, the number of mitoses in the cellular trophoblast of animals injected with the alkaloid was extremely high, whereas in the syncytial nuclei no mitoses could be recognised at the moment of fixation. It seems reasonable, therefore, to suppose that the syncytial trophoblast in the cat, as in the bat (Wimsatt, 1945) and man (Hofbauer, 1905; Grosser, 1927; Jones and Brewer, 1935; Hertig and Rock, 1941; Hamilton and Gladstone, 1942 and Wislocki and Bennett, 1943), is derived exclusively from the cellular layer.

The labyrinth of the dog differs from that of the cat in that in the former the lamellae are elaborately branched and take the form of so-called "lobules" disposed more or less vertically (Fig. 15. 54 a). Each lobule is formed from the adjacent trophoblastic layers of two primary villi enclosing a matrix in which are situated the relatively thin-walled maternal capillaries. The giant decidual cells and the layer of cellular trophoblast are, however, lacking and the maternal capillaries are enclosed by a relatively thick layer of syncytial trophoblast. However, as can be seen in Fig. 15. 55 a, the foetal capillaries lie in bays hollowed out in the trophoblast, and at many places where the latter adjoins foetal capillaries it is exceedingly thin (though not quite so thin perhaps) as in the bear (*see below*); and as the maternal vessels also have thin walls, the intimacy of the two circulations at these points is very striking.

For comparison with the cat and the dog the histology of the "placental membrane" in the definitive labyrinth of a number of carnivores is portrayed in Fig. 15. 55. The basic facts are as follows:

In the brown bear (Fig. 15. 55 b) the placental labyrinth is formed of very distinct lobules, consisting as in the dog, of relatively thin-walled maternal capillaries surrounded by a thin layer of syncytial trophoblast. Adjacent to the latter are the allantoic capillaries, lying as in the dog, in recesses excavated in the trophoblast. Although many of the foetal capillaries are separated from the maternal tissues by thin syncytial plates, others appear to be directly applied to the interstitial matrix surrounding the maternal capillaries. The placenta of the bear thus appears to be structurally more efficient, as an organ of foetal nutrition, than either that of the dog or cat. In the ferret and other Mustelids (e.g. *Zorilla*), on the other hand, the walls of the maternal capillaries instead of being composed of flattened endothelium as in the dog, consists of greatly enlarged cells, more or less cubical in form and characterised by intense cytoplasmic basophilia (Figs. 15. 55 c, d). The trophoblastic syncytial covering of the lobule is thin, though thicker than in the bear, and between it and the maternal endothelium is a thin but distinct layer of

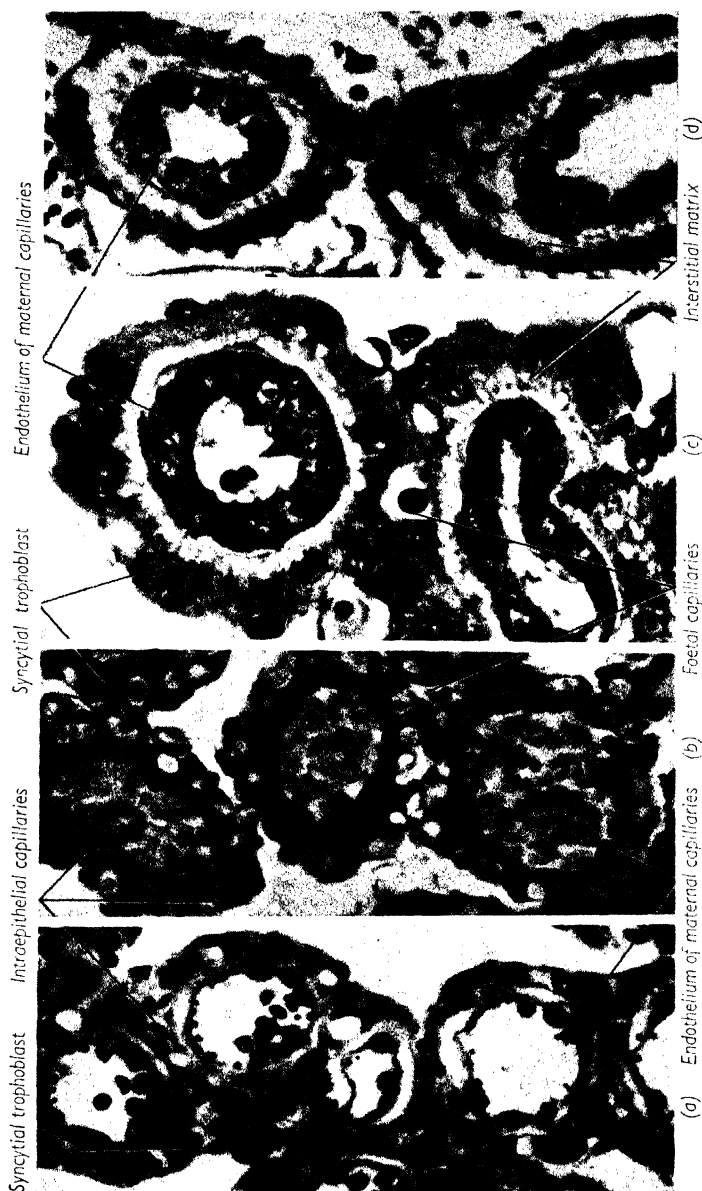


Fig. 15.55—Details of the placental membrane in a number of Carnivora.
 (a) Dog, $\times 455$. (b) Brown bear, $\times 231$. (c) Ferret, $\times 595$. (d) Zorilla, $\times 281$.

argyrophil fibrils. The foetal capillaries lie for the most part in the interspaces between the lamellae though some occupy bays in the syncytial trophoblast, as they do in the dog. It is noteworthy also that in the ferret, as is also true of the dog and cat (Wislocki and Dempsey, 1946a), alkaline phosphatase is present in dense concentrations in the interstitial matrix which intervenes between the

syncytium and the maternal cells, as well as in the outer part of the syncytial trophoblast itself.

From the foregoing summary of the histological structure of the labyrinth in the *Mustelidae*, it will be seen that the "placental membrane" bears a certain resemblance to that of the dog. However, in their possession of an endothelial lining consisting of greatly enlarged cells, the maternal capillaries of the *Mustelidae* (morphologically quite unlike true capillaries) differ markedly from those of the dog. If it be true, therefore, that changes in placental transfer rate can be correlated with structural changes in the placental membrane, it would be interesting to have data regarding the transfer rate of substances across the placenta in members of the two families, *Mustelidae* and *Canidae*. Here the use of isotopes may be expected to give important help.

The junctional zone.—The placental labyrinth is followed by an irregular junctional or transition zone, consisting of maternal cell-debris, the rich secretion

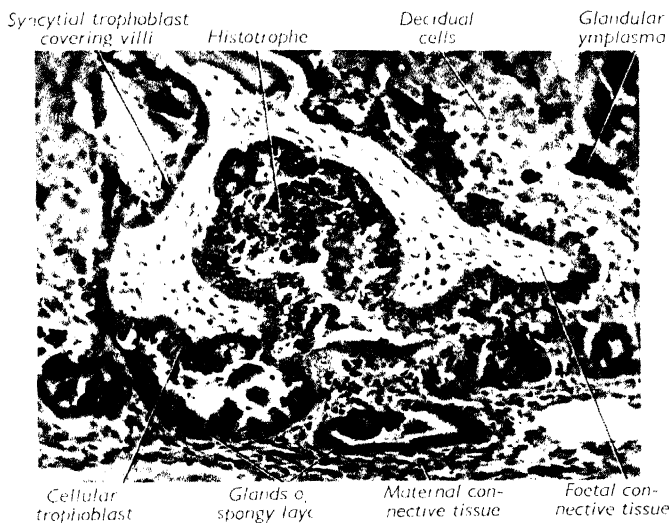


Fig. 15. 56— Junctional and deep glandular zone of the cat's placenta near term. $\times 130$.

of the uterine glands, leucocytes and the enlarged, terminal parts of the primary lamellae which occlude the mouths of the crypts (Fig. 15. 56). Decidual cells also occur in the junctional zone, and transitions between these and unmodified stromal cells; the decidual response is, however, less evident in the dog than in the cat. There is a certain amount of extravasated maternal blood mixed with the histiotrophe, the bleeding being attributable to the rupture of maternal vessels. This pabulum is evidently absorbed by the layer of vascularised cytotrophoblast covering the tips of the primary villi, the cells of which appear vacuolated, slightly enlarged and granular.

The deep glandular or spongy zone.—Immediately below the junctional zone is a spongy zone (the Drüsenkammer of German authors), consisting of the greatly hypertrophied portions of the uterine glands, whose thin walls appear very irregular.

The intervening connective tissue is scanty though richly vascular. The lumina of the glands are wide and filled with secretion and with detritic material resulting from the degradation of the surface of the glands. The material thus formed constitutes part of the transitional zone described above (Fig. 15. 56). The unaltered parts of the glands lie against the muscularis, and in the cat directly adjoin the spongy zone. But intervening between them and the dilated segments there is, in the dog, a thin subplacental layer (the Drüsendeckschicht) consisting of compact decidual cells through which prolongations of the glands of the spongy layer pass. Among the Carnivora, there are considerable variations in the degree of development of the spongy zone, the Fissipedia and the Pinnipedia differing sharply in this regard. In the cat and dog, for example, it is less distinct than in the sea lion (Fig. 15. 54 b) because the enlargement of the glands is less marked.

The central and marginal haematoma.—One of the characteristic features of the placenta of the Carnivora is the presence of "border" and "central" haematoma. Where these occur, the vascularised chorio-allantoic membrane develops villi which come directly in contact with maternal blood. In all the members of the order, larger or smaller maternal haemorrhages occur at an early period after the attachment of the blastodermic vesicle. The effusions vary in size and position. In the dog they occur regularly along the margins of the placental zone and the extensive breakdown of haemoglobin releases a green pigment, "uteroverdin," which accumulates in the characteristic "green borders" ("bordures vertes" of Duval); in addition, smaller haemorrhages take place into the substance of the placenta and form the "green pockets" or central haematoma, which may be isolated or joined to the green border by bridges. In the cat the haemorrhages occur in irregular positions and do not assume the green colour typical of the dog. On the contrary, the disintegrating maternal blood imparts a brownish hue to the region, hence the term "brown border" as applied to the marginal haematoma in the cat. Indications of the green border are present in the earlier stages but not in the completed placenta. In the otter and badger the effusion takes the form of a large, central blood-pouch, filled with a great variety of blood derivatives. In the ferret the conditions are similar; the main effusion occurs at the anti-mesometrial border of the uterus and divides the zone into two lateral discs, forming the so-called *zono-discoidal placenta* (Strahl). In *Zorilla* (Subba Rau, 1925) there are two localised haematoma at opposite margins of the placenta, whilst in the bear it is circular and limits the margin of the placental disc (see Fig. 15. 12). Marginal haematoma are said to be lacking altogether in the raccoon (Watson, 1881) and in the African civet (Strahl, 1905b). A thorough investigation of the haematoma has yet to be made (see, however, Janssen, 1933; Strauss, 1944).

According to Robinson (1904), the extravasated blood lifts the trophoblast from the uterine mucosa, and forces it in the form of irregular pouches toward the interior of the chorionic sac. Strahl (1890a, b), Bonnet (1902) and Subba Rau (1925) also state that the blood is effused between the mucosa and the allanto-chorionic membrane, and thus is contained in spaces whose walls are maternal on the one side and foetal on the other. Where the foetal tissue is in contact with maternal blood, the allanto-chorion is strongly folded and divides the area into the so-called "pockets." The surface of the folds is lined by columnar epithelial cells continuous at the placental margin with the trophoblast of the labyrinth. The ends of the ectodermal cells are expanded like clubs. Into

the meshes the constituents of the haematoma, unaltered erythrocytes and haemoglobin or its derivatives, are absorbed by phagocytosis (*see* Fig. 15. 14 *c*).

In the cat, supernumerary "pockets" are sometimes found in the form of "chorionic vesicles" that are entirely lined by trophoblast (Fig. 15. 57 *a*), and which in their gross shape and finer morphology closely resemble the structures found in the chorion of the Lemurs and of *Scalopus*. These vesicles are variable in size and are, as a rule, connected with the central haematoma, although occasionally they are associated with the brown border. They contain a bloodstained coagulum in which the extravasated erythrocytes become mingled with glandular

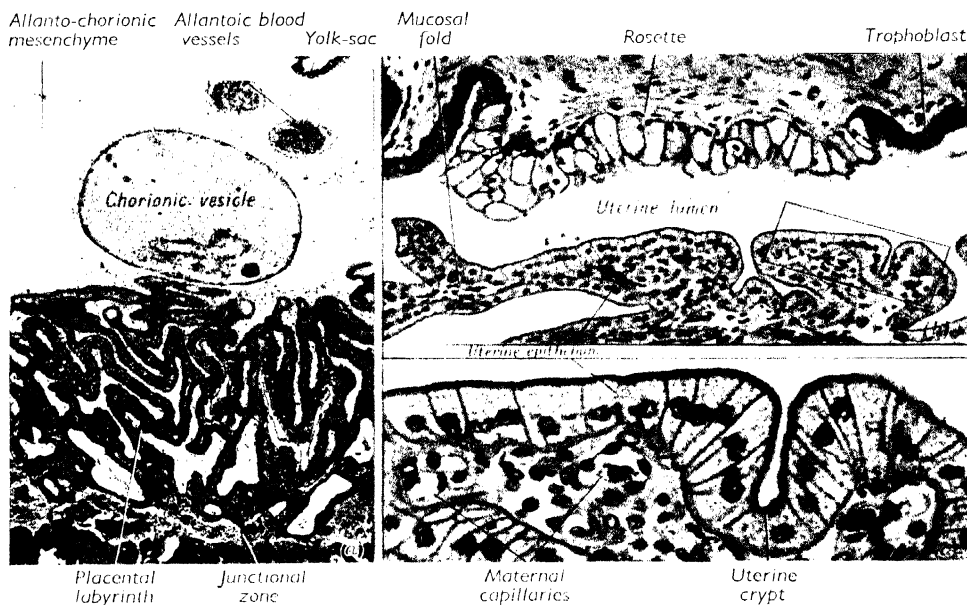


Fig. 15. 57—(a) Section of the chorio-allantoic placenta of the cat at 24 1/2 days showing a chorionic vesicle. 150. (b) Section through the paraplacental chorion of a bitch 8 weeks pregnant, showing the general relationship of a "rosette" to the cavity of the uterus and to the endometrium. 125. (c) Details of area enclosed in rectangle in Fig. 15. 57 (b).

secretion and disintegrating glandular epithelium. They are very vascular and their lining cells are extremely vacuolated, giving the impression of being concerned in the absorption of the uterine secretion filling their cavities. The walls of these structures may be smooth or provided with irregular bosses. Thus the observations on the chorionic vesicles of the cat support reasons adduced by Mossman (1939a) for supposing that they constitute an apparatus obviously similar to the marginal haematoma.

Besides the haematoma, the paraplacental chorion is sometimes provided with circumscribed, lightly staining, raised areas that are related to the mouths of uterine glands (Figs. 15. 57 *b, c*). These vary from single, swollen, columnar trophoblast cells to large accumulations over 100 μ in diameter. When a number of these pale staining trophoblast cells are grouped together they are arranged in the

form of a typical "rosette" and doubtless correspond to the irregular areolae of the pig and other ungulates. They have a look of permanence but we have no measure of how long individual ones may persist, though it is evident that these bodies are present on the dog's chorion at term. No mention has been found in the available literature concerning these bodies. However, it is hardly conceivable that they have been completely overlooked, since the foetal membranes of the dog have been the subject of active investigation for about 120 years, starting with Von Baer (1828).

Histochemical reactions of the placenta.

Sundry aspects of the histochemistry of the placenta of the cat and dog will be found in the papers of Bonnet (1902), Melissinos (1906), and Heinrichius (1889, 1891, 1914). Glycogen and fat have been described with great care by Dawson and Kusters (1944) for both pre- and post-implantation stages of the cat, while various cytological constituents, including fat, glycogen, alkaline and acid phosphatase, iron, ribonucleoprotein and phospholipids have been investigated by Wislocki and Key (1921), and Wislocki and Dempsey (1945, 1946a). The account as given here is based mainly on the observations of Wislocki and his associates as well as our own.

Fat is found in the intact surface and glandular epithelium after ovulation and before implantation, and in the lumen of the glands after desquamation of the cells. It appears in the epithelium partly as an infiltration and partly as a degeneration product of the cytoplasm. Fat is also exhibited in the trophoblastic lamellae of the placental labyrinth. It is present primarily in the syncytial layer and to a lesser extent in the cytotrophoblast wherever the latter is present. The lipoids which characterise the syncytial trophoblast give a strongly positive Schiff's reaction, show birefringency and exhibit a greenish fluorescence, properties which led Wislocki and Dempsey to suggest that the syncytium was the site of formation of placental steroid hormones. In the cat the maternal elements of the placenta are devoid of fat except in late pregnancy when the ageing decidual giant cells show signs of fatty degeneration. In the dog, on the other hand, fat is abundantly present in the endothelial cells lining the dilated maternal capillaries.

Glycogen and calcium are present in moderate amounts before implantation, and again shortly after implantation, in the surface epithelium and in the necks of the uterine glands (Dawson and Kusters, 1944; Wislocki and Dempsey, 1945, 1946a); but is altogether absent in the labyrinth throughout the greater part of pregnancy. Appreciable amounts of glycogen are found, however, in the endometrium between placental sites and in the secretion within the lumina of the uterine glands, whilst calcium accumulates in the giant decidual cells as term approaches. From these observations, and on the basis of their well-known hypothesis, that regions of sluggish metabolism are characterised by glycogen deposition, Wislocki and Dempsey concluded that the placenta of both the cat and the dog possessed an adequate circulation at all times. The accumulation of deposits of calcium in the decidual giant cells they interpret as a sign of senescence. The possible relation between phosphatase and glycogen in the pregnant uterus is discussed in Chapter 16, where also is reviewed the data of Cunningham (1920, 1922); Wislocki (1920); Pohl and Flexner (1941) and others regarding the passage of salts and vital dyes from mother to foetus and vice versa.

In the cat and other carnivores (e.g. dog, ferret), iron is limited to the extravasations of maternal blood which occur especially in the border and central haematomas, as well as in the blood-stained coagulum of the chorionic vesicles, and in the haemorrhages of the junctional region. None is found in the labyrinth or in the surface and glandular epithelium. In other mammals (pig, rodents and man), iron is concentrated and secreted by the uterine glands, so that in the carnivora, more than in other groups of mammals, iron appears to be transmitted to the growing foetus by means other than the uterine glands.

The histochemical reactions of the yolk-sac and the distribution of alkaline and acid phosphatase have already been alluded to (*see p. 219*).

The course of the blood flow in the cat's placenta.

The vascular pattern of the labyrinth of the cat and dog was first described by Tafani (1886, 1887) from injections of the placenta. From these observations and on the basis of personal findings, the course of the blood flow through the placenta of the cat may be stated briefly as follows: The principal maternal arteries emerge from the uterine musculature at regular intervals and traverse the spongy and junctional zones to enter the labyrinth. Here they assume a relatively straight course and proceed with but slight branching to the foetal placental face (Fig. 15. 52) where they break up into numerous and progressively smaller, horizontal tributaries before opening into large, confluent lacunae or sinuses. From these lacunae, whose endothelial walls are enclosed by a thin layer of trophoblast, the maternal blood is carried back to the foetal-maternal junction in a net-work of tortuous, dilated channels enclosed in the lamellae. At many places in the region of the junctional zone, these efferent vessels open into large, irregular, venous sinuses which convey the blood to the veins in the muscularis. The large maternal vessels entering the placental labyrinth are enclosed in a thick, trophoblastic sheath which extends almost down to the layer of uterine muscle, and in ordinary histological preparations the impression is gained, that they subdivide the labyrinth into roughly rectangular areas (*see* Fig. 15. 52). The large maternal veins emerging from the labyrinth to enter the muscularis do not possess special sheaths of trophoblast like the arteries.

In contrast to the maternal vessels, the umbilical arteries branch in the allantoic mesenchyme upon the surface of the placenta before penetrating the labyrinth. The penetrating branches, while still fairly large, extend nearly to the tips of the primary lamellae before breaking up into smaller capillaries which convey the blood through the labyrinth back to the surface. These efferent, allantoic vessels underlie the trophoblastic covering of the lamellae and often lie surprisingly close to the maternal vessels. Thus the pattern of the circulation in the cat's placenta is essentially the same as that characterising labyrinthine placentae in general, for here the foetal blood in the allantoic capillaries, embedded in the mesenchyme between the lamellae, flows in the opposite direction to that of the maternal blood in the dilated uterine capillaries of the lamellae.

Comparison of placentation in Ungulata and Carnivora.

From the preceding account, it is clear that certain resemblances and certain differences exist between Carnivora and Ungulata in the composition of the histotrophe. The most notable difference in the endotheliochorial placenta is the

absence of large amounts of the "uterine milk" which arises from the glandular secretion and the transudation of lymph. In Carnivora the gland secretion in the sub-placental area appears to be less important. Though the deeper part of the glands which lie in the spongy layer may secrete, the epithelium of the more superficial parts proliferates, and then degenerates and loses its secretory function; finally it forms a symplasma which plugs up the lumen of the glands. In the paraplacental region, it seems likely that the intact surface and glandular epithelium provide an important source of nourishment for the embryo from the beginning to the end of pregnancy (Figs. 15. 14 *a, b* and 57 *b, c*).

On the other hand, the amount of nutriment furnished directly from the maternal blood is increased. It is found in the extravasations already described and as individual blood corpuscles and droplets of haemoglobin or its derivatives in the lumina of glands. Leucocytes are found during the whole of pregnancy, but in less abundance than in the sheep. They do not act to the same extent as store-houses of fat, but some of them, the *siderophores*, contain granules which give an iron reaction. In the course of pregnancy they disappear comparatively early, with the exception of a few in the deep glandular layer.

In the Carnivora the foetal ectoderm of the area of placental attachment attacks the uterine mucosa more strongly than in the Ungulata. As a result, the maternal tissues, with the exception of the septa containing the placental vessels, disappear down to the middle of the spongy layer, and the tissue which is destroyed serves as pabulum for the developing embryo. In the cat the connective tissue cells form large, decidual cells before their final destruction, some of which become incorporated between the lamellae of trophoblast.

As in the Indeciduates, there is strong evidence that the trophoblast is actively phagocytic and takes up, as it meets them, the constituents of the pro-chorion, and later the degenerated tissues and extravasated blood. In the neighbourhood of the extravasations active absorption is indicated by the changes in shape of the trophoblast cells and by their pigmentation. In the mesoderm of the villi and its vessels no trace is found of any of the formed elements of the histotrophe, a proof that they undergo further transformation in the trophoblast after absorption.

The interchange of oxygen and carbon dioxide apparently occurs in the labyrinth, as in the cotyledons, of the sheep. Here only is the foetal circulation brought into close proximity with circulating maternal blood. Other foetal waste products are probably also got rid of in the labyrinth. Nolf (1896) suggests that the excretory products may be responsible for the degeneration of the maternal tissues into a symplasma.

In how far the other substances necessary for the growth of the embryo are taken up respectively from the circulating blood by purely physical or physiologically selective processes, and from the extravasated blood effusions by direct phagocytosis, is discussed in Chapter 16.

Placentation in the Sloths.

The Edentata are all known to possess a deciduate placenta (Turner, 1873; Strahl, 1906a, 1913; Fernandez, 1915; Newman and Patterson, 1910; de Lange, 1926; Wislocki, 1926, 1927, 1928a, 1928b; Heuser and Wislocki, 1935). There are, however, considerable differences in the finer placental structure, at least, in

the sloths (*Bradypodidae*) on the one hand, and the ant-eaters (*Myrmecophagidae*) and armadillos (*Dasypodidae*) on the other; in the former we find a placenta endotheliochorialis, while in the latter the labyrinth resembles that of the Primates and is haemochorial in structure.

In their possession of an endotheliochorial placenta, the tri-dactyl and di-dactyl sloths (*Bradypus griseus*, *Choloepus hoffmanni*) resemble the Carnivora just described, but differ markedly from the cat and dog in the complete absence of marginal and central haematoma, and in the insignificant rôle of the uterine glands in producing a secretion of nutritive value to the foetus. Moreover, in the Carnivora the allantois is voluminous, whereas in *Bradypus* it is vestigial and contained in a "body stalk" almost identical to that of the great apes and man (Heuser and Wislocki, 1935).

The foetal membranes and placenta of Bradypus griseus.

In the early stages of its evolution the placenta of the three-toed sloth is diffuse and multilobulate. The form of the placenta is, however, greatly modified during pregnancy and at the end of gestation forms a characteristic bowl-shaped mass occupying about one-third of the entire chorion and located in the fundic half of the uterus (Wislocki, 1927). As a rule, a membranous seam of chorion, opposite the insertion of the umbilical cord, divides it roughly into right and left halves.

The general shape and division of the ripe placenta is determined by the retrogression and degradation of the polar lobules (Wislocki, 1927). This is commonly held to begin in the cervical part of the uterus simplex where the lobules are especially well developed, and there is little doubt that it provides a ready and easily available source of histotrophe for the developing foetus. Moreover, Wislocki (1928b) has suggested that this widespread degeneration and resorption of the placental lobules may bear a remote relationship phylogenetically to the haematoma found in the Carnivora, particularly as they are encountered in *Cynogale*.

Microscopically, the labyrinth in *Bradypus* is formed of maternal and foetal vessels separated by trophoblast and a persistent fibrous stroma in which occasional spindle-shaped cells are encountered (Fig. 15. 58). Unlike the cat, however, the trophoblast is everywhere syncytial, no cytotrophoblast being recognisable in even



Fig. 15. 58—Details of the placental membrane of the sloth (*Bradypus griseus*) towards the middle of pregnancy. $\times 260$.

the earliest stages. Moreover, the development beneath the syncytium of a layer of epithelioid cells derived from the foetal stroma further distinguishes the sloths from the Carnivora. In *Bradypus*, as in the Carnivora, the capillaries of the foetal stroma lie in contact with the chorionic epithelium and ultimately come so near the surface that the cytoplasm of the syncytial net is reduced, in many places, to the thinnest membranes, thereby reducing still further the distance between the two blood streams. However, while this topographical arrangement provides for the readiest and easiest transfer of materials between the two circulations, it is noteworthy that the maternal capillaries are considerably enlarged and morphologically quite unlike true capillaries (cf. Figs. 15. 55 and 58).

According to de Lange (1926), the placenta of *Bradypus* represents an intermediate stage between the endothelio- and haemo-chorial types, the continuous layer of cells enclosing the maternal blood representing, he believes, the trophoblast. Wislocki's observations do not bear out this assumption, nor do our own. Personal observations, based on the microscopic examination of three placentae of this animal near term are in complete accord with those of Wislocki and indicate that the lining cells are exclusively of maternal origin. Moreover, the presence of remnants of maternal tissue between the lining cells and the trophoblast, alluded to above, might be regarded as evidence of a transitional stage from the syndesmo-chorial type to an endotheliochorial one. (See p. 211.)

Amniotic villi are present in *Bradypus*, but unlike those in the Ungulata, consist of a proliferation of the mesenchymal stroma of the amnion.

The early history of the yolk-sac in the sloth (*Bradypus griseus*) prior to the 3-somite stage is unknown. In embryos with 3 to 12 pairs of somites it is present as a spindle-shaped sac which extends from the body stalk across the coelom to the opposite wall of the blastocyst to which it is attached by a pedicle. After an initial early period of growth a portion of the yolk-sac becomes more and more tenuous until a mere thread-like connection remains which eventually breaks, separating the yolk-sac into two portions—a larger proximal part attached to the embryo and a small vesicular mass located on the opposite wall (Heuser and Wislocki, 1935). But, since the connection with the chorion is severed before the vitelline circulation is initiated, a chorio-vitelline placenta is not established. Subsequently the two fragments become completely resorbed. In embryos 27 to 36 mm. long, Wislocki (1927) found no remnants of the proximal fragment, but, in both specimens, remains of the detached chorionic segment could still be seen after the yolk-sac had shrunk away. A somewhat similar condition has been described in some early human embryos (Grosser, 1913, 1924).

Turner's (1873) and Wislocki's (1928b) descriptions of the placenta of the two-toed sloth agree in all essentials with that of *Bradypus*. In *Choloepus*, however, Wislocki has shown that the maternal perivascular tunic is better developed than in *Bradypus* and that the syncytiotrophoblast which intervenes between the maternal and foetal mesoderm is more completely reduced. Amniotic villi are not found in *Choloepus*.

In the Armadillos (*Dasypodidae*), early, complete inversion of the yolk-sac similar to that observed in the higher rodents occurs (Newman and Patterson, 1910), whereas the chorio-allantoic placenta presents an extraordinarily close resemblance to that of the great apes and man, and is of the haemochorial villous type (see p. 243).

The Placentation of Sorex (Shrew) and Talpa (mole).

Although Hubrecht (1893), Strahl (1892) and Vernhout (1895) regarded the definitive placental labyrinth of the *Soricidae* and the *Talpidae* as being haemochorial, like that of the *Macroscelididae* (van der Horst and Gillman, 1944), *Chrysochloridae* (de Lange, 1933), *Erinaceidae* (Hubrecht, 1889), *Tenrecidae* (Goetz, 1937; Strauss, 1943), *Potomogalidae* (Hill, 1938) and *Solenodontidae* (Wislocki, 1940), the investigations of Grosser (1927, 1936), Mossman (1937, 1939b) and Wimsatt and Wislocki (1947) have established fully its endotheliochorial character in the European mole (*Talpidae*), the common shrew, the mole shrew and the smoky shrew (*Soricidae*), and the tree shrew (*Tupaïidae*). Also, it may be convenient to the reader to be reminded at this point that in an American member of the *Talpidae* (*Scalopus aquaticus*) the placenta is epitheliochorial (Mossman, 1939a), while in *Centetes* (*Tenrecidae*) an accessory syndesmochorial "semi-placenta" supplements the main haemochorial region (Strahl, 1905b; see also p. 152).

In the shrew (Hubrecht, 1893), as in the Carnivora, the method of embedding is central and no decidua capsularis is formed. Unlike the Carnivora, however, the allantoic vesicle is small and transient. During development two types of placenta of increasing complexity have been described: a yolk-sac placenta and a chorio-allantoic placenta, both of which function concurrently during the whole of pregnancy.

Implantation and the yolk-sac placenta.

The pre-implantation changes in the uterus of the *Soricidae* have been described and figured by Hubrecht (1893), Brambell (1935), Brambell and Perry (1945) and Wimsatt and Wislocki (1947). The attachment of the blastocyst is modified, as in Ruminants, by special characteristics of the uterine mucosa. They differ from the uterine caruncles, however, in being proliferations of the surface epithelium. Before the fertilised ova reach the uterus, the mucosa undergoes preparatory, histological changes and there are variations in its thickness. It is thin at the mesometrial and antimesometrial segments, but greatly thickened over the sides to form two lateral cushions, in which the blood vessels are more numerous (Fig. 15. 59). According to Wimsatt and Wislocki (1947) the lateral folds are more prominent in *Sorex* than in *Blarina*. No glands are present near the mesometrium. They are collected on the opposite surface and open into a longitudinal antimesometrial groove.

When the blastocysts reach the uterus, which they do in *Blarina brevicauda* (mole shrew) about $4\frac{1}{2}$ days after the first mating (Pearson, 1944), further changes take place. Both the lateral regions increase in thickness by the proliferation of connective tissue cells and the formation of new vessels, while the antimesometrial part is widened out into a concave bell-shaped surface into which the glands open, thus giving the uterus a very characteristic appearance in early stages. (Figs. 15. 59 and 60.) The uterine epithelium proliferates, first over the lateral cushions and the mesometrial segment (Wimsatt and Wislocki, 1947), and later in the concave area. In the former, the proliferation reaches a considerable thickness as the new elements form a series of epithelial strands, of irregular width, which pass in among the cells and vessels of the deeper layer. In the course of development, certain of the epithelial columns become canalised, and in *Blarina* give rise

to crypts analagous to those which arise a little later in the mucosa of the anti-mesometrial region.

In *Sorex*, on the other hand, the nature of the epithelial reaction in the lateral and mesometrial regions is still not clear. Views on this subject were long dominated by the conclusion of Hubrecht (1893), that no crypts are formed in these areas; and a confirmation of this is given by Brambell and Perry (1945) who also failed to recognise crypts. However, in *Blarina*, as above stated, a strong case has

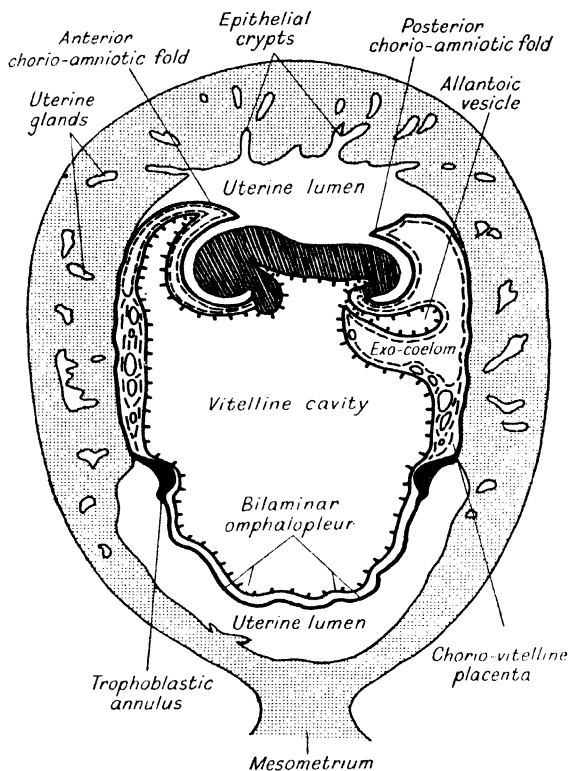


Fig. 15. 59---Diagrammatic representation of embryonic swelling of the shrew at the time of closure of the chorio-amniotic folds, showing the three functional subdivisions of the yolk-sac placenta.

been made out by Wimsatt and Wislocki (1947) for regarding the modifications in the epithelium of the lateral and mesometrial walls of the implantation chamber, as differing in no essentials from that occurring in the antimesometrial, allantoidean region; and the same may be true of *Sorex*. We must, therefore, conclude either that there is no general uniformity in the epithelial reactions in the *Soricidae*, or that some of the existing accounts of the subject are erroneous.

In the *Soricidae*, as in the *Carnivora*, the embryonic disc is oriented at the time of implantation towards the antimesometrial side and the blastocyst first attaches to the surface of the lateral, epithelial cushions by a zonyary strip against which the vessels of the area vasculosa spread out (Fig. 15. 59). Opposite the special areas of the mucosa the trophoblast thickens. In it two specialised regions are distinguishable, a region of attachment and, immediately adjacent to the latter mesometrially, a conspicuous thickened band of columnar

trophoblastic cells, "the trophoblastic annulus" of Hubrecht (1893) representing a morphological as well as a functional part of the bilaminar omphalopleur.

Below the annulus, the trophoblast of the non-vascular, bilaminar omphalopleur remains predominantly cuboidal and never becomes invasive; the endodermal cells are much smaller and between the two a Reichert-like membrane, consisting of collagenous material, appears. The latter is, in all probability, purely trophoblastic in origin. This is demonstrated, for instance, by Brambell and Perry's (1945) observation on *Sorex araneus* and *S. minutus*, or those of Wimsatt and Wislocki (1947) and Wislocki and Wimsatt (1947) on *Blarina brevicauda* and *Sorex*

fumeus; and is very probably true of Rodents as well (Wislocki, Deane and Dempsey, 1946). This makes the cytotrophoblast a truly remarkable layer, differentiating outwardly into syncytium and inwardly into collagen without the preliminary production of mesenchyme.

In the region of attachment, cell outlines in the epithelium of the cushions are lost, and a symplasma is formed. At the same time the trophoblast of the supra-annular segment becomes syncytial, is fused to the uterine symplasma, and slowly invades and erodes the maternal, epithelial crypts but sparing the maternal endothelium. In this way a chorio-vitelline placenta is formed, which functions as the principal, placental organ during the first half of pregnancy, but declines in importance as the extension of the exo-cœlom diverts its blood supply into the inverted roof of the yolk-sac (vascular splanchnopleur). In the *Soricidae* the yolk-sac is large and it undergoes extensive inversion with the formation of complex, splanchnopleuric villi.

The retrogressive changes also result in the absorption of the syncytial layer of the chorio-vitelline placenta as well as the epithelial thickening of the mucosal cushion. The disappearance is brought about by the combined action of the trophoblastic annulus and by a newly-formed sheet of trophoblast, first described by Hubrecht (1893), and later designated the "Trophoblastic curtain" (Brambell and Perry, 1945), which grows down from the upper rim of the trophoblastic annulus and adheres against the maternal tissue (Fig. 15. 60). The degenerated products of the thickened, uterine epithelium, the trophoblastic curtain (giant cells) and of a blood extravasate, which constantly exists between the annulus and the epithelium, are absorbed and transmitted through the endoderm to the yolk-sac. From it the vessels of the area vasculosa, which at this time reach their full development, carry the nutriment to the developing embryo. The annular haematoma is believed to arise from the destructive action of the trophoblastic curtain on the maternal capillaries, and, as in the Carnivora, provides the principal source of supply of iron to the growing foetus.

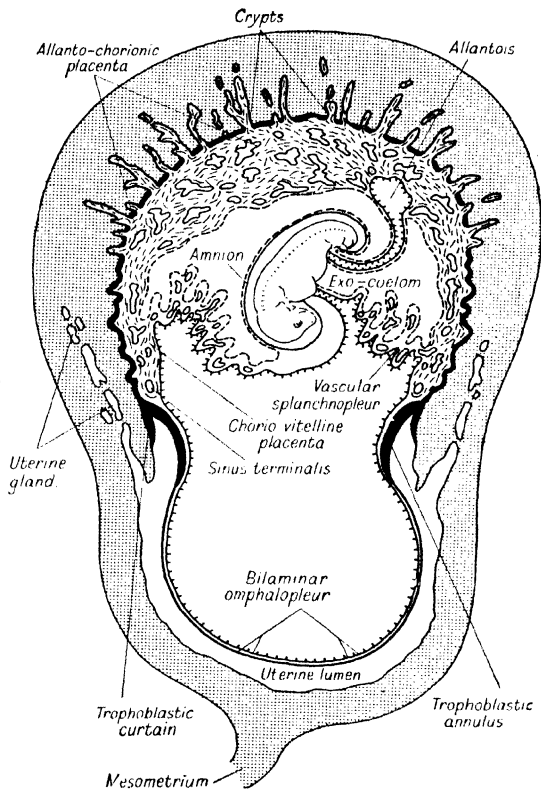


Fig. 15. 60 Diagram of an embryonic swelling in the shrew to show the vascularisation of the chorion by the allantois.

At about the time the chorio-vitelline placenta has reached its greatest development, the mesometrial border of the endometrial cushion begins to be undercut and becomes separated from the deep mucosal tissue (Fig. 15. 60). Accordingly the placental area becomes flanked by a wedge-shaped segment of the lateral mucosa which terminates in the mass of necrotic tissue and blood extravasation in the uterine lumen. This tongue-shaped strip of decidual tissue is interpreted by Brambell and Perry (1945) as an incomplete or vestigial decidua capsularis and may find its counterpart in the paraplacental capsule of decidual tissue described by Wislocki (1940) in *Solenodon*. With the advance of pregnancy this decidual strip is gradually absorbed.

Chorio-Allantoic placentation.

The chorio-allantoic placenta is a deciduous, discoidal, labyrinthine placenta of the endotheliochorial type. It is a simple disc with no undercutting folds of trophoblast or uterine epithelium. The allantoidean trophoblast is applied against the bell-shaped proliferation on the antimesometrial side of the uterus, and is fixed by solid buds of cytotrophoblast (primary villi of Hubrecht) which sink into the newly-formed crypts. Where the latter are located, the uterine epithelial cells are arranged radially like a fan, and later the internal parts of the cells break away and leave a crypt.

After destroying the epithelial lining of the crypts, the villi soon acquire a core of vascular, allantoic mesenchyme. Shortly thereafter, the cellular covering of the villi becomes syncytial, and the syncytial trophoblast gradually surrounds the hypertrophied walls of the capillary vessels in which the blood of the mother circulates. Concurrently, the persistent masses of cellular trophoblast between the bases of the villi proliferate to form thickened pads of intensely, basophilic cytotrophoblast which invest the ends of the intervillous maternal septa and doubtless play an important rôle in the synthesis of protein during this period of reconstruction. Subsequently the syncytial trophoblast thickens to a considerable extent by increment from the cytotrophoblastic pads (Fig. 15. 60), and in it the foetal mesenchyme, the cells of which are hypertrophied and curiously transformed, continues to branch and form secondary and tertiary villi. There is no penetration on their part into the decidual tissue between the crypts, but the maternal part of the placenta as a whole is gradually absorbed by the syncytial layer, and is replaced by foetal elements. As pregnancy advances most of the cytotrophoblast becomes converted into syncytial trophoblast, and at various points where this has occurred, the allantoic vessels are closely applied to the proliferated layer of foetal trophoblast which now surrounds the hypertrophied, maternal vessels.

In the late stages of the placenta the syncytial trophoblast exhibits signs of degeneration, and where it adjoins foetal capillaries it often becomes so attenuated that only the thinnest strands of syncytial cytoplasm separate the foetal vessels from the uterine capillaries, thereby establishing the same succession of layers between the two blood streams as are encountered in the definitive placenta of any of the Carnivora.

In *Talpa* the method of embedding is central and a simple chorio-vitelline placenta is present for a time. The chorio-allantoic placenta is discoid and is placed anti-mesometrially. The glandular secretion is of importance for the nourishment of the developing foetus during the greater part of pregnancy (Strahl,

1892 ; Vernhout, 1895). *Talpa* thus agrees with *Sorex* in respect to its mode of implantation and the localisation of the placenta. In contrast to *Sorex*, however, the yolk-sac is small and without villi, whereas the allantoic vesicle is of medium size and persistent.

At the beginning of pregnancy the mucosa shows variations in its different parts. Near the mesometrium, for about one-third of the circumference of the lumen, the glandular layer is thin (Fig. 15. 61). Antimesometrially the muscular layer is not

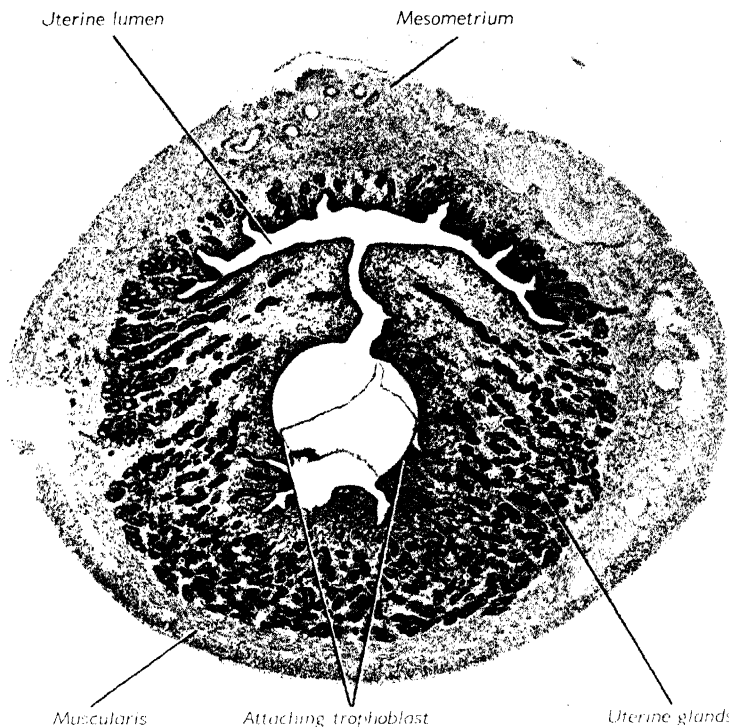


Fig. 15. 61 - Mole (*Talpa europaea*). Transverse section of an embryonic swelling with an implanting blastocyst. Note that attachment is first effected by an equatorial zone of the blastocyst wall. 33.

so well developed, but superficially to the glands there is a proliferation of connective tissue cells, through which the ducts run to open into the lumen. The first attachment is in this region.

The uterine horns show a series of small swellings where the ova are present. The blastocysts grow to a comparatively large size, and completely fill the lumen. They come in contact first with the two lateral areas of the antimesometrial segment by means of a thickened strip of trophoblast against which the vitelline vessels spread out to form the chorio-vitelline placenta (Fig. 15. 61). By their further growth the epithelium near the mesometrium (ob-placental region) is also destroyed and replaced by trophoblastic cells, which do not, however, penetrate

into the connective tissue or form villi. Hence this secondary mesometrial attachment forms a simple, non-vascular, bilaminar yolk-sac placenta. Unlike the chorio-vitelline placenta, it persists throughout pregnancy.

On the opposite side the decidual formation proceeds and the mucosa becomes thicker. In its substance a rich network of blood capillaries is developed. The epithelial cells lose their boundaries and form a symplasma. According to



Fig. 15. 62—Mole (*Talpa europaea*). Early stage in the differentiation of the chorio-allantoic placenta. $\times 140$.

Strahl (1892) this remains and forms the syncytial covering of the future villi. Vernhout (1895), on the other hand, has described a proliferation of the trophoblast with the formation of an endothelium-like layer which absorbs and gradually replaces the endothelial lining of the maternal capillaries. This behaviour of the trophoblastic cells is, however, denied by Grosser (1927, 1936), Mossman (1939b) and others, who interpret the placenta of *Talpa* as being fundamentally similar to that of *Sorex* and of the endotheliochorial type (Fig. 15. 62).

In the placental region the glandular epithelium is not changed, and around each opening a small area of the surrounding uterine epithelium persists. Over each gland opening the trophoblast forms a dome as in Ruminants. In the cavity between a gland orifice and the trophoblastic cap lies a dark secretion, pigmented by admixture with extravasated blood, and the cap is similarly pigmented. Hence the secretion is probably absorbed by the ectoderm throughout the greater part of

pregnancy during which the glands remain. After the disappearance of the surface epithelium at the point of connection with the blastocyst, the syncytial trophoblast penetrates into the connective tissue symplasma, and surrounds maternal capillaries. It is followed by the cytotrophoblast and the allantoic mesoderm. In the labyrinth the circulating maternal blood supplements the nutriment supplied by the glandular secretion. At birth the allantoic capillaries are withdrawn from the placental trophoblast, which remains behind to be absorbed by the leucocytes of the mother. This arrangement is known as "contra-decuate."

X. PLACENTA HAEMOCHORIALIS

The haemochorial condition represents the maximum expression of the invasive activity of the trophoblast, that is to say, it is characterised by immediate contact between the circulating maternal blood and the epithelium (trophoblast) of the chorion; histotrophe plays only a minor rôle, and nutrition becomes possible largely by processes similar to those occurring in the tissues of the body itself. There are many known cases where the main chorio-allantoic placenta is of this type (*see* Table I). In some haemochorial types, however, the trophoblast itself seems to disappear from much of the placenta, and where this occurs only foetal endothelium intervenes between the two blood streams. This arrangement is known as *haemoendothelial* (Mossman, 1926).

As was pointed out in discussing Grosser's classification (p. 147), the haemochorial condition is represented by two modifications—*labyrinthine* and *villous*, distinguished by the structure of the spaces in which the maternal blood is contained. In the former, well illustrated by the conditions found in some Insectivora, the Lagomorpha, most Rodentia,¹ many Chiroptera, the Hyracoidea and the Sirenia etc., the maternal blood spaces are narrow and capillary-like and the proliferating trophoblast over a limited portion of the wall of the blastocyst becomes slowly converted into vascularised lamellae by the ingrowth of mesoderm carrying the foetal blood vessels. For this type of placenta, Mossman (1926, 1937) and others have shown that quite generally, maternal arteries pass through the labyrinth to the foetal surface of the placenta, and the maternal capillary-like spaces into which they drain, run to the base of the placenta, whilst the foetal blood stream flows in the opposite direction (*see* p. 256 and Fig. 15. 43). However, besides the nourishment of the foetus through the establishment of a labyrinthine circulation, there is considerable reason to suppose that secretion provides an important source of nourishment for the embryo from the beginning to the end of pregnancy. In the villous type, on the other hand, histotrophe is less important and the trophoblast with its associated mesoderm is commonly described as growing out in the form of villi into the endometrium, producing the placenta by the gradual destruction of the maternal tissues and by the opening up of wide, confluent, intervillous spaces through which maternal blood flows. Thus in the definitive placenta it is possible to distinguish (1) a basal plate formed by a layer of decidua with a variable amount of associated trophoblast, (2) a chorionic plate, (3) the villi which originate from the chorionic plate and may terminate freely in the intervillous space or may be attached to the basal plate (*see* Figs. 15. 83 and 86). Placentae of this type have been described in the scaly-tailed squirrels, *Anomalurus perci* and *A. erythronotus*,¹ the spotted hyena, *Crocuta*, and in the armadillos among the Edentata (Newman and Patterson, 1910; Strahl, 1913; Fernandez, 1915). However, as is apparent from the accounts of Strahl (1903), Wislocki (1932, 1933b) and Hill (1932) the anthropoid apes and man exhibit the highest expression of the *haemochorial villous* condition.

In order to compare the manner of differentiation of the trophoblast in the two types of haemochorial placentae, it will be sufficient to describe certain phases of

¹ For details of the haemochorial villous condition in Rodentia, *see* Branca and Cretin's (1925) account of the ripe placenta of *Anomalurus perci*.

placental growth and differentiation in a few representative orders. The Lagomorpha, Rodentia and Primates have always been favourite objects for the study of these changes and may, therefore, be conveniently taken as types.

Lagomorpha and Rodentia.

In connection with the development of the haemochorial and haemoendothelial conditions in these orders it must be remembered that there are variations in the mode of attachment. For example, in the Lagomorpha (hares and rabbits) it is *centric* or *superficial*, while in the Rodentia, it is *eccentric* in the Sciuromorpha (squirrels and gophers) and Myomorpha (rats and mice), and *interstitial* in the Histricomorpha (guinea-pigs).¹

Correlated with the conditions of implantation in these forms we find differences in the histological details of the formation of the placenta, but in all the ultimate form of the placenta is discoid and the endometrial relation deciduate. The placenta type is usually labyrinthine and the class, on the basis of finer structure haemochorial, probably becoming haemoendothelial when mature.

It was in Rodents that the proliferation and vascularisation of the trophoblast were first described by Selenka (1883). Later Duval (1889-1891) gave a fuller account of the earlier stages, and Hubrecht (1889) discovered the same conditions in other orders.²

Placentation in the Rabbit

The detailed histology of the placenta of the rabbit has been admirably worked out by Duval (loc. cit.), Maximow (1898, 1900); Chipman (1903); Mossman (1926) and many others. The account of it as given here is based, as in the previous edition of this book, mainly of Chipman's monograph and is supplemented by the observations of Mossman as well as by personal findings on a series of pregnant uteri ranging in age from the sixth day of pregnancy to near term.

Preimplantation phenomena.

The fertilised ova of the rabbit, each enveloped by the zona pellucida and a homogenous sticky layer of albumen, reach the uterus at the beginning of the fourth day (72-74 hours) after coitus (Cruikshank, 1797; Assheton, 1895; Gregory, 1930). At first they have no fixed position, but by the seventh day, when the blastocysts are about five millimetres in diameter, they have become equally spaced along the uterine lumen and produce well-marked localised swellings. The investment formed by the zona pellucida and the albumen layer (prochorion) is very thick while the fertilised egg is still in the Fallopian tube; on the fourth day when the uterus is reached it rapidly thins but persists up to the end of the first week of pregnancy (Assheton, 1895). On the eighth day the zona disappears and the first organic contact of the embryonic and maternal tissues is established (Fig. 15. 63).

¹ Complete interstitial implantation is also observed in man, gorilla, chimpanzee, hedgehog and in certain bats, e.g., *Glossophaga soricina*. See also Chapter 14.

² For Insectivora, see Grosser (1927), de Lange (1933), Goetz (1937), Hill (1938), Wislocki (1940); Cheiroptera, Van Beneden (1888, 1899), Branca (1927), Wimsatt (1945); Hyracoidea, Wislocki and Westhuysen (1940); Sirenia, Wislocki (1935b).

As is well known the follicular phase of the cycle in all mammals is accompanied by characteristic changes in the uterine mucosa. The "mature" uterine membrane of the non-pregnant rabbit already shows specialised structures which are of importance for the attachment and nutrition of a future embryo. These consist of symmetrical pairs of longitudinal folds, first described by Hollard (1863), and subsequently named by Minot (1890); *placental* folds, the largest, situated one on each side of the groove corresponding to the insertion of the mesometrium; *ob-placental* folds, the smallest, opposite the mesometrium; *peri-placental* folds, intermediate in position and size. Each fold is divided by transverse fissures

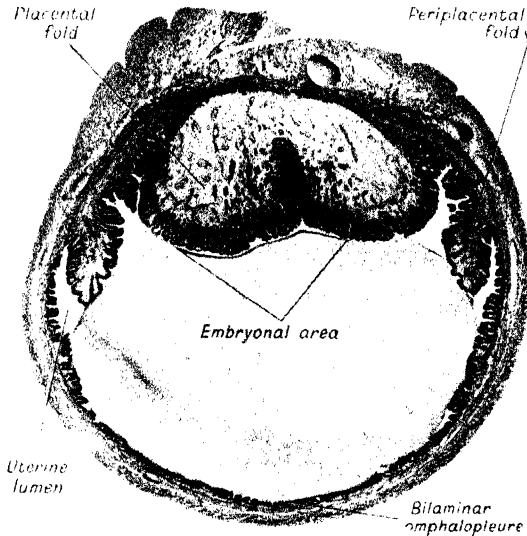


Fig. 15. 63.—Transverse section of an 8-day gestation sac of the rabbit. The placental folds are large; the muscular walls of the sac are thin. 7½.

into rectangular areas, the *cousinets* of Hollard. With the onset of pregnancy two of these areas on the placental folds, placed one on either side of the mesometrial or intercotyledonary groove (Duval) hypertrophy and form the maternal part of the future discoid placenta (Bischoff, 1842), which is thus bi-lobed (Fig. 15. 63). The folds of the mucosa are essentially increased areas of the mucosal connective tissue, but they differ from the caruncles of Ruminants in having glands.

Implantation and the yolk-sac placenta.

On the entrance of a fertilised ovum into the uterus, the folds especially the ob-placental, become shortened, and a localised cavity appears which is occupied by the blastocyst. At the same time there is a marked hyperplasia of the cellular connective tissue of the placental and peri-placental folds leading to a thickening of their bases (Chipman, 1903). By the sixth day, the capillaries are also increased in these regions. In the ob-placental folds appear enormous giant-cells (Monster cells, Minot) derived, according to Chipman, by a process of "degenerative hypertrophy" from the epithelium of the surface and glands. Hammond (1917), however, suggests that they may be detached cells from the trophoblast and is supported by Sansom (1927) who finds the giant-cells undoubtedly arising from the foetal tissue. Loveland, Maurer and Snyder (1931) have shown that these cells do not react to glycogen stains, a finding testifying, it is stated, to their origin from the foetal ectodermal wall.

In the rabbit placenta, the various cells which are involved in the origin, storage and release of glycogen have been carefully studied by a number of workers and especially by Chipman. From their observations there is abundant evidence that the storage of glycogen is a conspicuous function of decidual cells of the rabbit

placenta and is not evident in the epithelial tissue of foetal origin. These giant-cells of the ob-placental region attain their maximum development at about the seventeenth day (Fig. 15. 64 c) and have disappeared by the twenty-sixth day (Muraoka, 1919).

In the rabbit the mesoderm begins to spread out from the embryonic region about the end of the first week of gestation, and it gradually reaches half-way round the circumference of the blastocyst. Near the embryo appears the area vasculosa. This vascular mesoderm remains in close approximation to the trophoplast until the extension of the exo-coelom separates them, after a relatively brief period of existence, into non-vascular extra-embryonic somatopleur or chorion, and extra-embryonic splanchnopleur or vascular yolk-sac (Figs. 15. 8 and 9). The mesoderm splits into two layers over its whole extent in the rabbit, and it is limited below by the sinus terminalis, which communicates with the vitelline veins. The lower half of the yolk-sac (bilaminar omphalopleur) is non-vascular, whereas the adjacent mucosa is richly supplied with circulating maternal blood.

The non-vascular yolk-sac and the chorio-vitelline placenta.

The first thickening and specialisation of the trophoblast, other than that of the ectoplacenta (*see* below), occur in the region of the "bilaminar omphalopleur" and the "area vasculosa" opposite the ob-placental and peri-placental lobes respectively, and it is by them that the blastocyst obtains its first attachment over its lower pole (Assheton, 1895). This is noticeable when the embryo has reached about the 8-somite stage. Even before actual contact has been made, as judged by microscopic sections, a profound change begins to take place in the opposing uterine mucosa. Both the surface and glandular epithelium begin to swell, vacuoles appear and the cells fuse into a symplasma with the nuclei crowded away from the bases of the cells. The thickened areas of cellular trophoblast insinuate processes into the uterine symplasma and the connections thus established constitute regions of bilaminar yolk-sac and chorio-vitelline placentation (Figs. 15. 64 a, b). Doubtless they provide pathways through which a substantial part of the early placental exchange takes place and through which much of the liquid which accumulates in the rapidly enlarging blastocyst must pass.¹ The striking thing about the bilaminar omphalopleur in this species is its temporary character. Though very large at first, it begins to degenerate about the tenth day and by the seventeenth day, except for remnants near the sinus terminalis and the trophoblastic mononuclear giant-cells, has disappeared altogether. The chorio-vitelline placenta is devascularised about the gill-slit stage.

The inverted yolk-sac placenta.

Co-temporary with the spread of the exo-coelom, the extra-embryonic splanchnopleur, i.e. the vascular portion of the yolk-sac, becomes inverted into the yolk-sac cavity, partly as a result of the accumulation of albuminous fluid in the extra-embryonic coelom and partly, perhaps as a result of the growth of the embryo. In consequence, the yolk-sac cavity becomes reduced to a narrow cleft

¹ Since the above was written it has been shown by electrophoresis and the ultracentrifuge that albumin, α , β , and γ globulins and fibrinogen are all present in the yolk-sac fluid of 7- and 8-day embryos, in similar proportions to each other as in the maternal plasma (Brambell, Hemmings and Rowlands, 1947).

and the endoderm of the vascular part of the yolk-sac is brought into close proximity with the endoderm of the non-vascular part (bilaminar omphalopleur). With the disintegration of the bilaminar omphalopleur, the inverted splanchnopleuric segment of the yolk-sac comes in contact, through its endoderm, with the uterine lining, and in this way forms the foetal constituent of the inverted yolk-sac placenta which persists till term (Fig. 15. 64 c). The inverted yolk-sac placenta of the rabbit is of the complete type in contrast to the incomplete type seen in the squirrels where the bilaminar omphalopleur never disintegrates.

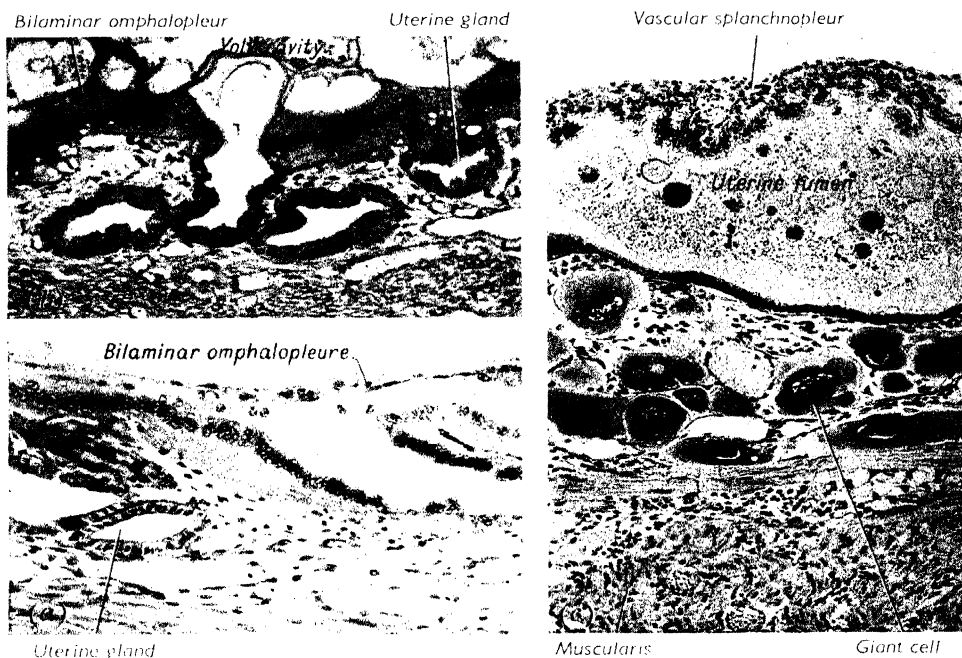


Fig. 15. 64 --Rabbit. (a-b) Sections through portions of ob-placental region showing changes in the endometrium associated with the attachment of the bilaminar omphalopleur. (a) Earlier. 110. (b) Later stage. 187. (c) Section of portion of antimesometrial wall of implantation chamber of an animal 16 days pregnant, showing inverted roof of yolk-sac (vascular splanchnopleur) and giant cells. 85.

Chorio-allantoic placentation.

The formation of the discoidal, labyrinthine, chorio-allantoic placenta in the rabbit is initiated at the time of implantation but its characteristics are not fully developed until about the twelfth day of pregnancy. Attachment to the placental folds is effected during the eighth day (Biedl, Peters & Hofstätter, 1921), and involves at first a horse-shoe-shaped area of the uterine epithelium. During the sixth and seventh days, the epithelial cells covering the free surface of the placental lobes proliferate and fill up the superficial crypts of the mucous membrane. The glands are as yet unchanged, and the increasing blood supply leads to a free secretion which is usually considered to be added to the albumen-layer, and then to be absorbed by the trophoblast. There is no appreciable transudation of lymph such as occurs in ruminants.

As the blastodermic vesicle grows, it presses against the folds and levels them. Hence at the time of attachment (eighth day), the surfaces of the placental lobes are nearly regular. The covering epithelium is thickened, the cells are greatly increased in size, cell boundaries have become indistinct, and the active proliferation of the connective tissue cells is continued to form the placental cotyledons. Between them is now a well-marked *intercotyledonary groove* (Fig. 15. 63). According to Chipman, however, "these cotyledons have increased in prominence not so



Fig. 15. 65—Portion of the placenta of a rabbit 8 days pregnant, showing first attachment of the trophoblast. Note the symplasma and the mitosis in the cellular trophoblast. *z. 170.*

much by their own growth as from the fact of the further resorption and shrinkage of the remaining portion of the mucosa." At the same time the zona disappears and the trophoblast proliferates in crescentic areas on either side of the embryonic disc, which is placed opposite the groove between the placental cushions, thus allowing room for the later formation of the amniotic folds over the embryo. The two crescents (the *ectoplacenta* of Duval) represent the area of attachment of the trophoblast and have together a shape not unlike a horse-shoe. They have been named by van Beneden, who first described them "*fer-à-cheval* placentaire."

Where the maternal and foetal tissues are in contact (Fig. 15. 65), the surface epithelium shows a form of degeneration similar to the epithelial symplasma of the endotheliochorial placenta of the Carnivora. The uterine epithelium swells and becomes symplasmic as the trophoblast approaches it. Soon it is compressed into a

thin layer between the trophoblast and decidua, degenerates, and finally disappears altogether being apparently absorbed by the cells of the trophoblast (Fig. 15. 65). The only feature about this early relationship which is not typical of an epitheliochorial placenta is the symplasmic nature of the uterine epithelium. With the complete disappearance of the epithelium the trophoblast comes in contact, on the ninth day, with the connective tissue of the uterus and exhibits for a time a syndesmochorial relationship (see Table II). For a further discussion of the interactions of the trophoblast and endometrium at the time of implantation, see Mossman (1937), also Wislocki and Streeter (1938).

The swelling of the epithelium is even more marked in the glands and their

proliferated epithelium forms a symplasma which obliterates the lumina and even dilates the gland many times beyond its original size (Fig. 15. 65). This process extends into the necks of the glands and, in rodents, usually involves the entire gland (cf. the similar but less marked phenomena in the Carnivora, where the bases of the glands persist as active secretory vesicles). At these points the trophoblast advances more quickly, as if the resistance were weaker, and the line of attachment is undulating (Fig. 15. 66). The uterine glands are, however, only temporary pathways to the invasion of the trophoblast. They eventually disappear completely and are replaced on the tenth day by multinucleate decidual cells. The dips thus correspond to the gland orifices and represent the beginnings of the so-called villi.

The blood vessels are large and numerous and have no adventitia, i.e. they are wholly capillaries (dilated arterioles). But the more deeply placed of them acquire an adventitia, the *perivascular sheath* (Masquelin and Swaen, 1879). It is formed of one or two layers of large connective tissue cells which represent the first appearance of the glycogen containing decidual cells. They are seen first at about the ninth day of pregnancy when the trophoblast is first definitely attached to the endometrium. Initially they surround the endometrial arterioles. Subsequently they begin to appear around the veins, and by the time the allantoic circulation is established the endometrium in the sub-placental region has become "transformed into closely packed perivascular sheaths, which can be spoken of in its entirety as the decidua basalis" (Mossman, 1937).

The allantoic outgrowth first appears in embryos of 9-somites and rapidly extends through the exo-coelom until, at the tenth day, it comes into contact with the outer wall of the blastocyst over the site of the future placenta. Subsequently the area of attachment is extended as the vascular body of the allantois



Fig 15. 66—Portion of the placenta of a rabbit 8 days 11 hours pregnant, showing the thickened ectoderm attached to the placental lobe and dipping more deeply at the position of the glands. The figure also illustrates the pronounced cytoplasmic basophilia of the syncytial trophoblast which in places is enveloping the maternal capillaries. · 165.

flattens against the surface of the attaching placental trophoblast. The small marginal zone of the chorion not invaded by the allantois is the so-called "chorion laeve."

After the destruction of the superficial and glandular epithelium, the trophoblast advances into the interglandular tissue, the cells of which degenerate in turn and are absorbed. The advance is most rapid where a capillary is met with.



Fig. 15. 67.—Surface of placental lobe in a rabbit 10 days pregnant. Notice the thin lamina of syncytial trophoblast enclosing the maternal blood. 165.

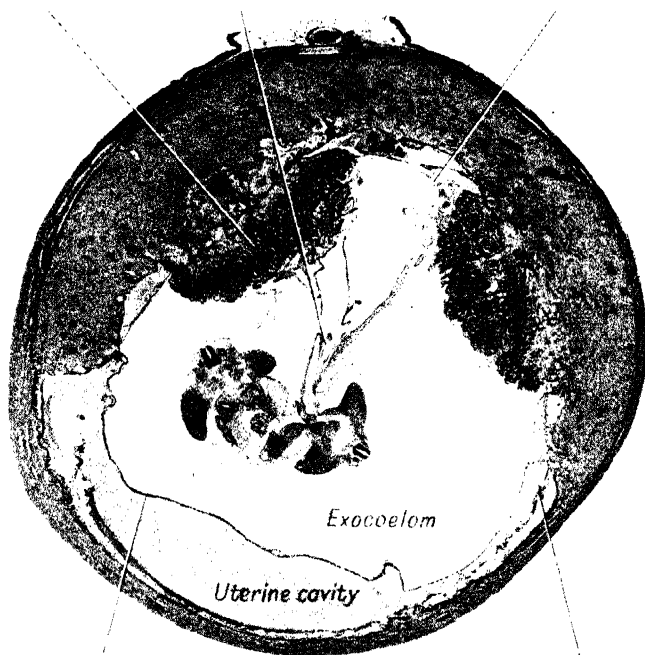
presence of degenerating maternal tissue and foetal syncytial lamellae enclosing spaces filled with maternal blood (Fig. 15. 67).

The mucous membrane is now differentiated into two zones, the *intermediary region* (zone of ingrowth) and the *region of the uterine sinuses* (Duval). The intermediary region lies superficially. It is closely packed with fusiform stroma cells and capillaries with thin perivascular sheaths of *uninucleate* decidual cells. In the region of contact with the invading trophoblast and presumably by the influence of the trophoblast, the decidual cells increase in size and become *multinucleate* with as many as 30–40 nuclei (Maximow, 1898, 1900). They lose their perivascular position and pervade the whole of the region. In their midst run the maternal

The rapidly ingrowing processes of the trophoblast are more or less lamelliform and "they push in until they reach the endothelium of the capillary, then gradually working round its wall they completely circumvent the vessel which, albeit retaining its endothelial wall, now lies in the midst of tissue of foetal origin." The trophoblastic lamellae thus temporarily exhibit an endothelio-chorial condition. These changes may be observed during the second half of the ninth day, but by the tenth day the maternal capillaries which appear completely surrounded by the trophoblast have entirely lost their endothelium and lie, as maternal blood spaces, in the midst of the trophoblastic lamellae. With the disappearance of the endothelium we see the haemochorial condition established. The placental zone formed in this way—by ingrowth of the trophoblast—is termed the "zone of ingrowth" (Dürchdringungszone, Grosser, 1927), and is characterised at first by (1) the absence of foetal mesoderm, and (2) by the

vessels with simply a thickened endothelial wall. At the same time all traces of the gland ducts are lost, the cells of the latter appearing to serve as pabulum for the decidual cells. In the region of the uterine sinuses the blood vessels dilate to form large spaces and the decidual cells remain uninucleate till a considerably later period. The junction between the two zones is marked by the blind ends of the glands which are filled with degenerated epithelium. In section each appears as an island of glandular symplasma.

Placental cotyledon Allantoic vessel Mesometrium Intercotyledonary groove



*Inverted wall of yolk-sac
(vascular splanchnopleur)*

*Remnants of bilaminar
omphalopleur*

Fig. 15. 68—Cross-section of uterus, embryo and membranes of a rabbit 12 days pregnant. 6.

The placental trophoblast is now at the tenth day differentiated into two layers, the superficial syncytiotrophoblast and a deeper cellular layer, the cytotrophoblast. The latter disappears before the end of pregnancy. Processes of vascular mesoderm invade the trophoblast at intervals, and break it up into columns so that the ectoplacenta as a mere thickened ectodermal lamina ceases to exist. At the same time the foetal tissues continue to advance and surround maternal capillaries, the endothelium of which they replace. These vessels lose at once their original size and shape and become distorted into large irregularly spaced cavities, the sinuses. This is the first contact of the trophoblast with the maternal blood and the tissue separating the two blood streams is foetal endothelium, strands of mesenchyme, cellular trophoblast and thin syncytial trophoblast (Fig. 15. 67).

By the twelfth day the placenta has become divided into an inner trophoblastic or foetal region, an outer uninucleate region, and an intermediate multinucleate region, and owing to the continued growth of its lateral portions, the foetal component becomes kidney shaped with a hilum of maternal tissue. The general disposition of the placenta—its two cotyledons, and the foetal and maternal



Fig. 15. 69—A portion of the chorio-allantoic placenta at the stage shown in Fig. 15. 68. $\times 65$.

portions of these—can be gathered from Figs. 15. 68 and 69. The two regions of the latter are easily distinguishable. Moreover, the intercotyledonary groove has opened out so that the surface of the placental lobes face each other.

In the rabbit the trophoblastic columns are hollow tubes which surround the maternal blood channels; they are closed on the foetal side and open on the maternal side and their cavity is filled with maternal blood. This is an important point, since the placenta is built up from these units and many of its structural

features, such as the direction of the maternal and foetal blood flow through it, are dependent upon this method of formation. Subsequently the arrangement becomes more complex, each hollow column being divided up into a series of



Fig. 15. 70—Cross-section of a portion of the 15-day rabbit placenta. $\times 60$.

hollow tubes parallel to the original column, and each tube in turn forming a series of hollow tubules.

The endothelium of the maternal capillaries frequently ruptures just before it is overtaken by the foetal tissue, and irregular blood extravasations are formed

and later, surrounded by trophoblast. In the deeper layers of the intermediary region, according to Chipman, capillary haemorrhages occur more slowly, and give rise to a fibrinous tissue with red and white blood corpuscles scattered through it. This is similar to the haematogenous symplasma of Bonnet. It gradually increases in amount and extends to the region of the uterine sinuses. While the trophoblast advances along the vessels, it remains stationary at the non-vascular parts. Hence there is an interlocking of maternal and foetal tissues, and by the fourteenth day peninsulae of multinucleate cells come to lie between the projections of the trophoblast. At the same time the intermediary region decreases in thickness and the trophoblastic lamellae reach the superficial sinuses of the deeper zone (Fig. 15. 70). Here the uninucleate, decidual cells again become multinucleate, apparently at the expense of the blood symplasma, in the same manner as formerly at the expense of the glandular symplasma. We agree with Mossman (1926) that at no time are the walls of the uterine sinuses composed "solely of fibrin lamellae." According to Chipman the sinuses enlarge and their walls proliferate into several layers of cells, which after mid-pregnancy are gradually replaced by laminae of fibrin. The deepest region of the uterine sinuses, that next to the musculature constitutes now a definite zone along which separation of the placenta takes place at parturition, and it is called the "zone of separation."

Once these developments have occurred, the placenta may be said to possess its full tissue complement. Subsequent differentiation involves (1) an increase in size primarily effected by proliferation of the mesenchymal and vascular constituents of the foetal labyrinth and an increase in number, size and complexity of the maternal blood channels, (2) a change in shape of the placental cotyledons, and (3) the gradual elimination of two constituents of the placenta that are conspicuous during the earlier phases of its development (a) the cytotrophoblast and (b) the syncytiotrophoblast.

In the rabbit the partitioning of the trophoblastic columns into long tubular compartments is begun on the fourteenth day and occurs, not in definite steps, but as a continuous and irregular process of subdivision, so that various sizes of tubes (Fig. 15. 71) are found from soon after their inception up to a few days before term (Mossman, 1926). Chipman (1903) has described two stages in the development of these vessels from the parent column. In the first stage bud-like ingrowths into the main lumen of the column, i.e. into the maternal blood spaces, proceed from the syncytial layer of the trophoblast. These unite with similar ingrowths from opposing walls and, maintaining a direction which is in general parallel to the parent wall, divides the latter into a set of tubes. These syncytial processes have a cellular core and the vascular mesoderm pushes in along their cellular axis. In this way the parent column is divided into a set of parallel tubes and from these, by another subdivision, the smaller vascular channels, the tubules are formed. Such a set of tubules is called by Duval a "lobc." A tube is thus simply a small maternal blood channel of the syncytiotrophoblast. At each division the thickness of the foetal tissue between the maternal blood in the axis and the foetal vessels decreases, till finally there is only a film of trophoblast and the vascular wall. At places, as has been shown for the twenty-second day by Duval (1890), Chipman (1903), Lockhead (1922), Mossman (1926) and others, the trophoblast even is wanting, and the foetal endothelium alone intervenes between the two blood streams thus reducing the placental barrier from a haemo-

chorial one to an haemoendothelial one. The question of the placental barrier in rodents is discussed more fully by Mossman (1926, 1937); Hard (1946) and Wislocki, Deane and Dempsey (1946).

The prominence of the lobes of the twelve-day placenta as represented in histological sections persists to about the twenty-fifth day of pregnancy. However, subsequent expansion of the gestation sac, as a result of the growth of the foetus and the concomitant enlargement of the placental lobes, cause the latter during the next two days to become flattened and disc shaped, but the disc is less extensive than the typical disc at full term.

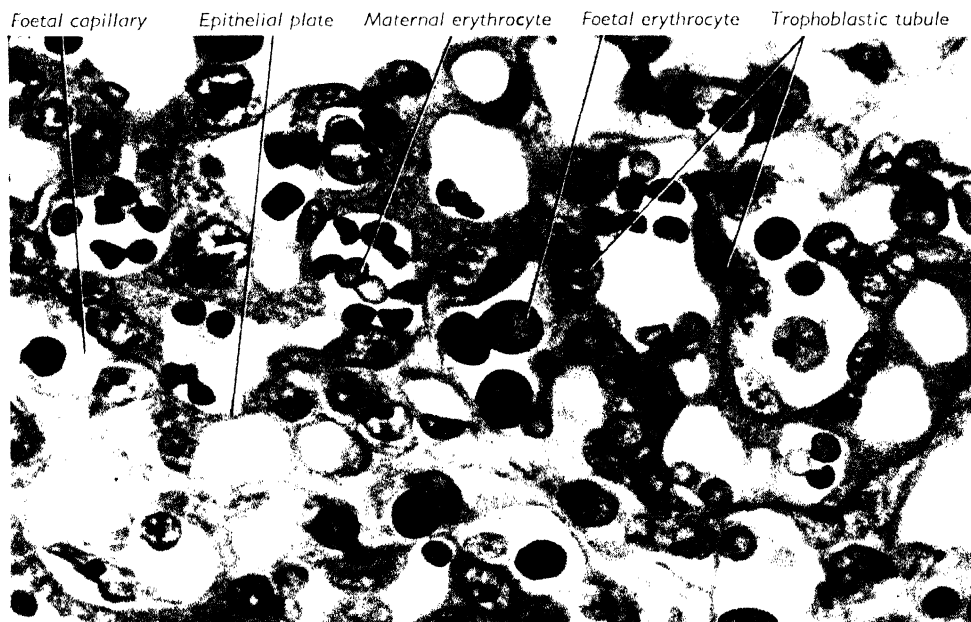


Fig. 15. 71. Section of 17-day rabbit placenta in trophoblastic region showing maternal and foetal corpuscles (nucleated) and relative thinness of placental membrane. $\times 920$.

The histology of the placental membrane in the labyrinth of the seventeen-day rabbit placenta is portrayed in Fig. 15. 71. The centre of the photo-micrograph shows foetal vessels containing large nucleated red corpuscles, and about them are clustered a number of anastomosed, trophoblastic tubes containing smaller, maternal erythrocytes. The foetal capillaries nearly fill the meshes of the foetal labyrinth adjoining the syncytiotrophoblast. Now, although many of these capillaries are separated from the maternal blood by substantial "epithelial plates," at many places where it adjoins the foetal capillaries, the syncytiotrophoblast is exceedingly thin and it is very difficult to decide whether the tissue separating the blood streams in the lobes consists, in every case, of both syncytiotrophoblast and endothelium. Very likely it does, but of this we are not certain. However, the absence of argyrophil fibres from around many of the capillaries of the foetal labyrinth at these sites make it seem very probable that, as early as the

seventeenth day, there are places in which only foetal endothelium separates the blood in the maternal tubules from that in the foetal capillary. These regions in the rabbit placenta present, therefore, the most intimate approximation of maternal and foetal circulations. The progressive disappearance of the syncytiotrophoblast continues to the end of gestation and accordingly in the definitive placenta a true haemoendothelial relationship obtains within the organ. The disappearance of the cytotrophoblast occurs during the twelfth and thirteenth days.

During the second half of pregnancy the uterine epithelium of the ob-placental and peri-placental regions where it is destroyed, is regenerated from the epithelium lining the persistent deeper portions of the uterine glands. The regenerated epithelium may even spread inwards for a short distance below the margin of the allantoic placental disc, so that the latter acquires a constricted base. At birth this stalk of connection is torn and after parturition the contraction of the uterus so reduces the abraded surface that the latter is hardly discernible with the naked eye. Accordingly, the uterus is almost immediately ready for a new pregnancy. At the end of pregnancy the maternal placenta consists almost entirely of blood and blood symplasma, except for a thin rim of tissue containing blood sinuses at the zone of separation. In the rabbit parturition is practically bloodless. The gestation period is from thirty to thirty-two days.

The course of the blood flow in the rabbit placenta.

Chipman does not state whether the maternal blood circulates in the trophoblastic tubes, but Maximow (1900) says that it does. Similarly Duval (1890) says "The maternal blood *circulates* from the foetal extremity towards the maternal extremity of a lobule" (i.e. the series of tubules derived from one tube). According to Masius (1889), "the maternal blood circulates in an ectodermal mass of foetal origin."

Mossman in an important paper of 1926 has shown "that in the rabbit placenta at all stages between the eighth and twenty-eighth day of gestation the maternal blood circulates through definite arterial and venous channels having peculiarities which readily differentiate them one from the other." Moreover, the foetal and maternal blood streams, instead of coursing parallel to one another, actually flow in opposite directions. This pattern of circulation of maternal and foetal blood through the placenta is essentially the same as that characterising labyrinthine placentae in general.

According to Mossman, the principal arteries enter the maternal placenta on the mesometrial side of the gestation sac and penetrate at once to the foetal side. Here they break up over the surface of the placenta into numerous and progressively smaller tributaries which are continuous with the trophoblastic tubes that carry the blood back through the lobes towards the maternal placenta. At many places the trophoblastic tubes reunite to form larger, efferent vessels which merge into venous channels that penetrate the zone of separation and unite with veins of the uterine wall. The umbilical arteries, instead of penetrating the placenta to the opposite surface before breaking up as do the maternal arteries, branch in the allantoic mesenchyme above the placenta and upon the surface of the placenta before penetrating the disc. The penetrating branches of the foetal vessels enter nearly as far as the deepest growths of the trophoblast into the maternal tissue. Here they split up into smaller branches and capillaries by which the

foetal blood is returned through the lobes back to the surface. These capillaries are closely packed between the trophoblastic tubules and are more or less parallel to them. In this way the maternal and foetal blood of the tubes and capillaries of the labyrinth flow in inverse directions, the maternal blood from the foetal surface of the placenta towards the uterus, the foetal blood from the uterine side towards the maternal side. Thus the foetal blood has the opportunity to come into equilibrium, not with maternal venous blood but with that at the arterial end of the maternal capillary bed. This mechanism is illustrated diagrammatically in Fig. 15. 43 and is discussed in Chapter 17.

A comparison of placentation in the Lagomorpha and the Carnivora.

As compared with the placenta of Carnivora, it is obvious that the dilatation of maternal vessels is much more marked in the rabbit, and throughout the placenta the maternal blood is in direct contact with foetal tissue and not only at the marginal or central haematoma. The blood is not degenerated to a sufficient extent to exhibit the varieties of pigmentation found in the zonary placenta. In the rabbit the maternal blood circulates in an ectodermal mass of foetal origin. Herein lies a great difference between the placentae of Rodentia and Carnivora or Ungulata. In the sheep nutriment is furnished by the glands; the maternal blood which is in contact with foetal ectoderm is stationary and small in amount, and serves chiefly as a supply of iron; the exchange of gases takes place through foetal and maternal tissues. In the cat and dog the gland secretion is said to be less important; the blood is again stationary and restricted to certain situations, and it shows markedly degenerative appearances, but it is greater in amount, and probably furnishes other substances besides iron for the foetus; in the placental labyrinth the maternal blood circulates and here the exchange of gases is effected, but again both maternal and foetal tissues intervene between the two blood systems. In the rabbit the glandular secretion is still less important after attachment, and even the blind ends do not secrete; throughout the placenta there is normal circulating maternal blood in direct contact with foetal tissues, and it serves both as nutriment and for the exchange of gases. In addition there are stationary blood extravasations which are engulfed by the trophoblast, but they are subsidiary. In the dog, the cat and the rabbit there is marked formation of symplasma which may be connected, as Bonnet suggested for the dog and Maximow for the rabbit, with the slowing of the circulation in the placenta, or may be the result of a trophoblastic influence.

In the placenta of the rabbit there is one other difference which marks it off from the placenta of the Carnivora (not cat) and links it with the Insectivora and man---the connective tissue cells of the mucosa form decidual cells. They assist to an important degree in the preparation of nutriment for the embryo. They exercise a phagocytic action on the neighbouring degenerated maternal tissues, glandular remnants and fibrin, and so attain their greatest development, while, at the same time, they become store-houses of foetal nutriment. At a later period they degenerate and are absorbed by the trophoblast. Their possible function as a protection against the attack of the foetal ectoderm has been denied. At the end of pregnancy their defence is no longer required, as the trophoblast has also lost its activity.

*Placentation in the Mouse and Rat**Implantation.*

The fertilised ova of the mouse reach the uterine cavity on the third day, and segmentation is completed one day later (Lewis and Wright, 1935). Soon after the eggs have entered the uterus the zona pellucida disappears, independently, it would seem, of any ferments or other specific conditions of endometrial origin (Fawcett, Wislocki and Waldo, 1947), and fixation of the ovum to the uterus can be quickly attained. The mouse produces a large litter of young. An average litter of 7.4 with a range of 2 to 12 is reported by Gates (1925), and similar figures are given by Watt (1934). The second litter is said to be the largest, most births taking place between 4 P.M. and 4 A.M. The duration of gestation is about 19 days varying from 18 to 20 (Asdell, 1946).

Implantation is eccentric and each blastocyst comes to rest in an anti-mesometrial groove or crypt. By this time the blastocysts are evenly spaced along the long axis of the uterine cornu, and exhibit in addition, a remarkable constancy in their orientation within the uterine lumen (Widakowich, 1911; Mossman, 1937). At first spherical, they become ovoid on the sixth day, with their long axis perpendicular to the long axis of the uterus. One pole is turned towards the mesometrium and is composed of several layers of cells, whilst the opposite pole is single-layered. It is nourished by the glandular secretion, and perhaps also by a transudate, in which, however, leucocytes are not present (Fig. 15. 72). In the rat,

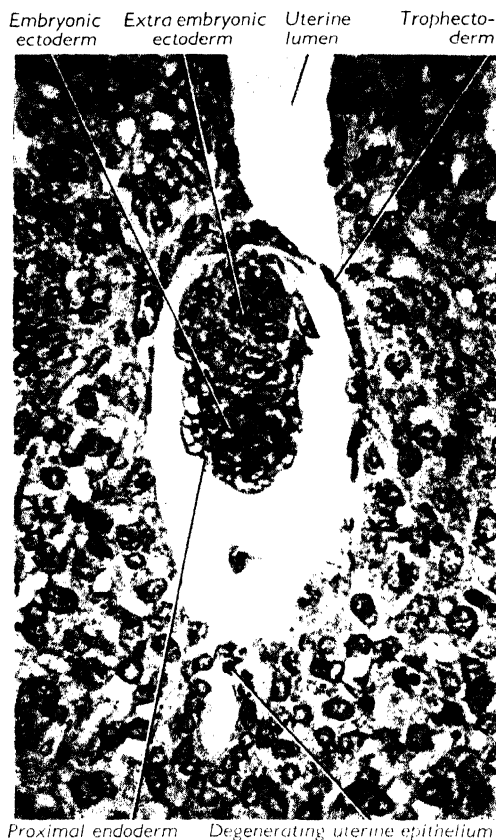


Fig. 15. 72—Longitudinal section of implanting egg cylinder of the mouse at 4 days 16 hours after mating. Observe the uterine epithelium above, and below the blastocyst; at the sides it has disappeared. 385.

of uterine polarity, by reversal of the mesometrial-antimesometrial axis of the uterus, does not interfere with the establishment of this normal embryonic axis; for regardless of the position of the mesometrium, the embryos are invariably implanted with the inner cell-mass correctly oriented, though reversed in relation to an assumed gravitational axis.

The connective tissue of the mucosa shows a thickening at the point where a blastocyst rests. The epithelium degenerates as the result of contact with the

foetal ectoderm (Duval, 1891), or of pressure by the oedematous and thickened subepithelial connective tissue layer, which interferes with the nutrition of the epithelium (Burckhard, 1901; Mossman, 1937). The epithelium degenerates by becoming itself oedemic. In the cells, the chromatin clumps on the inner surface of the nuclear membrane, the cell boundaries disappear, and a symplasma is formed which later becomes broken up into nuclear and cellular fragments. Fat globules, which are present in the epithelium of the non-pregnant uterus, are found in the detritus and also in the foetal ectoderm. Glycogen is present also in the secretion within the uterine cavity as well as in the uterine epithelium and in the stroma beneath.

With the destruction of the epithelium appears the first sign of decidual formation. The connective tissue cells increase in size and displace the glands; the capillaries dilate irregularly, and at places form sinuses (*see* Krehbiel, 1937).

On the sixth day the *ectoplacental cone* (Duval, 1891) is formed by a proliferation of the ectoderm at the mesometrial pole of the blastocyst. This thickening is the precursor of the foetal portion of the placenta. It plugs the opening between the crypt and the lumen of the uterus. At the same time the lips of the crypts are gradually brought nearer to each other by the swelling of the tissues, and at the end of the seventh day they fuse and cover the ectoplacental cone. In this way the blastocyst is completely shut off in a decidual cavity, the "Eikammer," from the uterine lumen (Fig. 15.73 a). The roof of the chamber forms the primary decidua capsularis and it is gradually thickened by the formation of decidual tissue. In it new blood vessels are developed and they form a specially thick network.

By this time the blastocyst has become tubular in shape, and it shows the so-called *inversion of the germinal layers*. In the earlier stage a cavity, the pro-amniotic cavity, appears in the inner mass of cells. The roof of the cavity becomes thickened to form the "Träger" (Selenka, 1883, 1884) or ectoplacental cone, which is at first cylindrical and later conical, with its base resting on the mesometrial pole of the blastocyst. By its inward growth it shoves before it the floor of the inner mass consisting of ectoderm and endoderm. In this way an invagination is produced in the tube with the ectoderm internal to the endoderm—the reverse of the condition found in most other chordates. Hence the germinal layers are said to be inverted.

The structures and relations of the egg-cylinder during the implantation period are portrayed in Figs. 15.73 a, b, c, and must serve as a background for the discussion of the details of placentation. The egg cylinder exhibits the form typical for rodents with inversion. Three cavities are now present in place of the single pro-amniotic cavity which it formerly contained. The most dorsal of these, the one nearest the mesometrium, is the false amniotic, or ectoplacental cavity, and is lined with extra-embryonal ectoderm. Its roof is continuous with the tissue of the Träger; its floor, together with a layer of mesoderm, the so-called "chorion" constitutes the roof of the extra-embryonal coelom. Its lateral walls are clothed externally with a layer of splanchnic endoderm (proximal endoderm, Snell), the visceral yolk-sac wall having, by now, extended upwards almost to the junction of the Träger with the lateral walls of the ectoplacental cavity. The middle and largest cavity is the extra-embryonal coelom, lined with mesoderm. Its floor, which is extremely thin, consists of two closely apposed



layers of ectoderm and mesoderm and forms the amnion. Its lateral walls are clothed with columnar endodermal cells, while its roof, as already described, forms the chorion. The most ventral cavity is the amniotic cavity, lined with ectoderm.

The egg cylinder itself is invested with a layer of visceral endoderm. Over the medullary plate it has the form of a single layer of ovoid cells (embryonal endoderm) while over the extra-embryonal ectoderm it consists of tall columnar cells. This layer gradually thins down as it approaches the Träger and before it reaches the latter it is reflected back as the parietal yolk-sac wall, which is represented by isolated cells, lying in contact with Reichert's membrane.

The decidual cavity is at first small and ovoid in correspondence to the shape of the blastocyst, and has a thick wall. As it grows, the lumen of the uterus is obliterated and at its point of contact, with the mesometrial wall, the epithelium of the latter disappears. Thereafter, the two layers fuse (at about 8 days), and at the point of fusion the placenta is developed. On each side of the decidual swelling the uterine lumen remains patent, but at this stage of development there is no continuous passage along the length of the uterus. The lumen of the uterus is later re-established at the floor of the decidual cavity, so that the new cavity passes ventral instead of dorsal to the embryo. Hence the primary decidua capsularis forms the basalis—which contributes to the formation of the placenta—and a secondary capsularis of multinucleate cells is formed which is recognisable till shortly before term. Between the basalis and the capsularis is the central vascula or intermediate parietal zone of the decidua (*see* Krehbiel, 1937).

According to Sabotta (1903) the antimesometrial lumen arises from the persisting basal segments of the uterine glands around the periphery of the decidual swelling. These enlarge and unite with each other and with the portions of the uterine lumen which persist between the decidual enlargements. In this way the blastocyst comes to lie in what appears like a simple thickening of the mesometrial wall of the uterine lumen.

Giant cells.—A characteristic feature of the placenta of rodents is the presence of numerous large mononuclear cells, Riesenzellen (Grosser and others), Megalokaryocytes (Jenkinson) or Giant cells as they have been called by various other authors. They occur abundantly in the mouse, rat, squirrel and vole. In the murine rodents they are especially large and thus very conspicuous, but nowhere do they seem to attain the number and dimensions that they do in the placenta of the water-vole (Sansom, 1922). In the mouse, giant cells first appear on the inner surface of the decidua around the anti-mesometrial pole of the blastocyst (Figs. 15. 73 *a*, *b*). They are phagocytic and migratory (Fawcett *et al.*, 1947). Duval (1891) stated that each was derived from a cell of the foetal ectodermal wall of the yolk-sac, and later from a cell of the ectoplacental cone. As they increase in number they form a distinct layer, two to five cells in depth, between the yolk-sac and the wall of the implantation cavity, and some wander into the decidua and lie singly or in groups. In their interior degenerating leucocytes are frequently seen. Sabotta (1903) also stated that they were foetal in origin and helped to fix the ovum and erode maternal capillaries. More recently Grosser (1927), Snell (1941) and Fawcett *et al.* (1947) have expressed agreement with the observations of Duval and Sabotta. The first two investigators state that in the rat and mouse, cells from the transitory trophoblast at the anti-mesometrial extremity of the blastocyst, after penetrating the mucosa, grow into primary giant cells which serve to enlarge the

implantation cavity by destroying the decidual cells which constitute its walls. A second series arises later from the mesometrial surface and margin of the trophoblast of the Träger and according to Grosser (1909, 1927) constitutes the "Umlagerungszone" or "Durchdringungszone." Mossman (1937) also attributes the origin of the mononuclear giant cells in rodents to foetal tissue, saying that in the more specialised ones (Heteromyidae), they arise very early and often make up the bulk of the so-called Träger. According to Wislocki, Deane and Dempsey (1946) alkaline phosphatase is abundantly present in these monocuclear trophoblastic giant cells at the base and margin of the foetal placenta. (Cf. the binucleate cells in the placenta of the sheep.)

In contradistinction to a foetal origin, Kolster (1903) and Pujiula (1908) have brought forward evidence from the histological appearance that they are transformed decidual cells, and this is strongly supported by Disse's (1906) investigations on the field-mouse in which the giant cells are found before the ovum has become embedded, and the first to appear are at an appreciable distance beneath the surface epithelium. Grosser (1927) believes that a certain number of giant cells are of maternal origin being formed from decidual cells which, under the influence of the invading trophoblast coalesce and form symplasma masses or maternal giant cells. In this he is supported by Krehbiel (1931) who finds that the decidual plasmodia or decidual giant cells in the Townsend ground squirrel are not single hypertrophied cells, but are the result of fusion of a group of cells of disintegrating tissue. Somewhat similar symplasia occur in the mouse, but the origin of these seems uncertain (Snell, 1941). Jenkinson (1902) also recognised two groups, but assigned to them different origins, foetal in the "Eikammer" and maternal in the decidua. Sansom (1922) agrees with Disse as regards the exclusive maternal origin of these placental giant cells but differs from him as to their time of first appearance and as to their origin. According to Sansom all the giant cells in the water vole (*Microtus amphibius*) are of maternal endothelial origin, and a similar origin is claimed by him for the giant cells in the placenta of the mouse. In the rabbit, Chipman (1903) finds the giant cells to be transformed epithelial cells of the uterine glands, whereas Sansom (1927) states that they are derived from the trophoblastic cells.

From the evidence reviewed in the foregoing paragraphs it will be realised that the precise origin of these giant cells is still in doubt, and while the majority are probably of trophoblastic origin, some at least may have a maternal derivation.

Although there still remains an element of uncertainty in regard to their primary origin, there seems no doubt at all that the giant cells are phagocytic and migratory. The tissue around them undergoes fatty degeneration, and in their interior may be seen remnants of connective tissue and endothelial cells and fat globules. Many capillaries are ruptured, and red and white corpuscles are absorbed. Such an absorption of maternal tissue by the giant cells leads to an increase in the size of the implantation cavity and a thinning of its wall (Disse, 1906; Grosser, 1927). Moreover, the giant cells have long cytoplasmic processes which interdigitate with one another to form a loose meshwork between Reichert's membrane and the decidua, the spaces of which are filled with circulating maternal blood (Everett, 1935). This vascular spongy tissue undoubtedly facilitates expansion of the embryo (Sansom, 1922), and constituting as it does the maternal portion of the yolk-sac placenta, provides, with Reichert's membrane, an important

seat of exchange for the nutrition of the foetus. In spite of their abundant supply of nutriment, the life history of the giant cells is short. There is evidence, however, that some of them may persist, for some weeks, that is, from one pregnancy to another (Mossman, 1937). No cell divisions occur; numerical studies of metaphase figures following colchicine injections have provided indirect support for this.

The increase in size of the implantation cavity is accompanied by a thinning of its walls, and when the uterine lumen has been reformed in the anti-mesometrial wall of the uterus, the yolk-sac splanchnopleur is only separated from the uterine cavity by a comparatively thin layer, consisting of Reichert's membrane, a network of attenuated and degenerating giant cells and a thin layer of circular muscle (Fig. 15. 74). As the embryo increases in size this giant cell network becomes stretched still further and its constituent cells become greatly reduced, until finally the capsularis is represented by a thin membrane composed of the now flattened cells of the yolk-sac splanchnopleur closely applied to Reichert's membrane. In the mouse (Jenkinson, 1902) and rat (Grosser, 1909, 1927), this layer ruptures some time before birth (15-16 days), with the result that the vascular endoderm of the yolk-sac becomes exposed to the uterine lumen. According to Duval (1891), the thinning of the wall of the implantation cavity is a mechanical process, since the cells do not increase in number, but it is probably more complicated involving, as we have already noticed, the activity of the phagocytic giant cells which appear around the ovum and on the inner surface of the decidua.

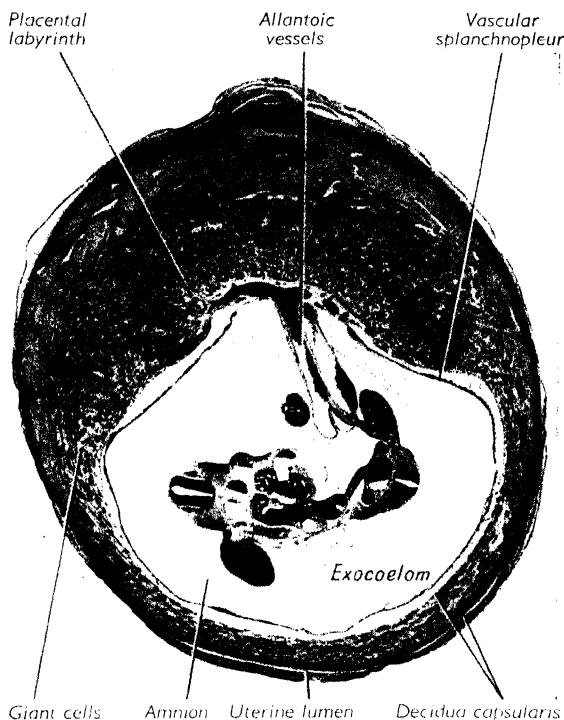


Fig. 15. 74—Mouse. Cross-section of uterus, embryo and membranes, showing formation of secondary uterine lumen and decidua capsularis. Age unknown, but probably about 10 days. $\times 13$.

Chorio-allantoic placentation.

The definitive chorio-allantoic placenta of the mouse is fundamentally like that of the rat and of other rodents. It is a deciduous, discoidal, labyrinthine placenta of the haemochorial type, probably becoming haemoendothelial near term. The ectoplacenta has a composite origin from two rudiments, the ectoplacental cone or Träger and the allantois. The Träger is at first cone-shaped in vertical section, but on the seventh day it thickens rapidly and grows forward

through the uterine tissues, losing, as it does so, its compact pyramidal form. During its invasion into the uterine tissues it destroys not only the decidua, but also the endothelial lining of the maternal blood vessels in the neighbourhood of the cone, and the blood extravasations thus formed become enclosed in trophoblastic tissue. Where it is in contact with the decidua basalis, it retains for a time its identity as a cellular cap and extending rapidly in the lateral direction increases its area of attachment to the maternal tissues (Fig. 15. 73 *c*). At the same time the blastocyst again becomes spherical and the extra-embryonal coelom, consequent upon the accumulation of fluid, increases markedly in size. As a result the lower wall of the ectoplacental or epamniotic cavity, the "chorion," is pushed upwards until it comes into contact and fuses with the overlying wall of

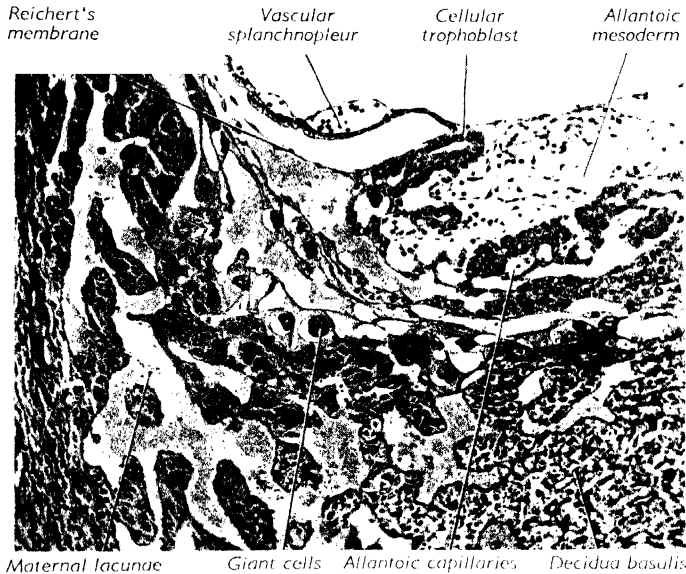


Fig. 15. 75---Margin of the chorio-allantoic placenta of the mouse at a stage somewhat earlier than that shown in Fig. 15. 74. For explanation see text.

the Träger to form the cap-like "couche plasmodiale compacte" of Duval, or "lamina" of Everett. Fusion is initiated over the middle region so that after a while a narrow circular cleft is all that remains of the former epamniotic cavity. When finally, this too becomes obliterated, the trophoblastic cells of the chorion become indistinguishable from those of the Träger. Before its fusion the "chorion" consists of a single layer of cuboidal cells invested on its under surface by an extremely attenuated layer of mesodermal cells.

While these changes are taking place, the spindle-shaped giant cells, around the lateral and antimesometrial wall of the implantation cavity enlarge rapidly and enclose within their meshes lacunae of circulating maternal blood (Figs. 15. 75 and 78). This belt of highly vascular tissue comes into intimate contact with the outer wall of the blastocyst, which now consists of the fine structureless membrane of Reichert derived from the original wall of the vesicle. The most internal of the giant cells are in organic continuity with the membrane, but their attachment here is

less firm than their connections with the neighbouring giant cells (Everett, 1935). The outermost giant cells are in contact with the maternal decidual cells many of which have, under the influence of the giant cells, undergone a symplasmic transformation. Sabotta (1903), Asai (1914), Jenkinson (1913) and Grosser (1927) regarded the blood in the lacunae formed by the communication of the spaces among the giant cells as stagnant and assigned to it an histotrophic rôle, particularly in the transfer of iron from mother to foetus. Everett (1935), however, has

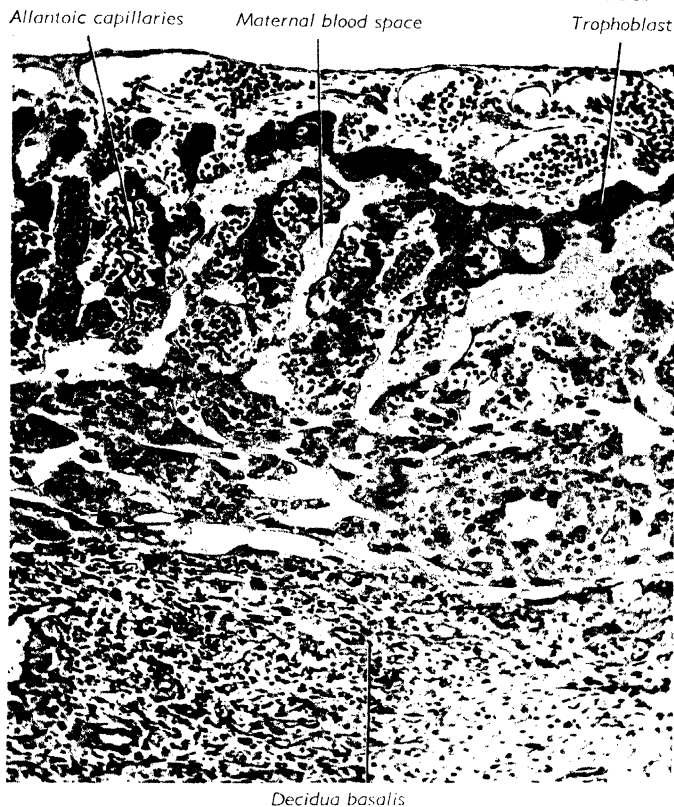


Fig. 15. 76--Early stage in the establishment of the placental labyrinth in the mouse. $\times 115$.

shown that it remains a part of the maternal circulation, and in the rat is renewed every 20 minutes. That this blood, during life, circulates through the meshes of this network was first suggested by Sansom (1922).

The allantois¹ in the mouse is a solid mass of richly vascular mesoderm with no endodermal cavity (Fig. 15. 73 b). Growing out from the posterior end of the

¹ There is a justifiable question as to the propriety of designating as "allantois" a body stalk which is believed never to contain any endoderm. Nevertheless, there may be some advantage in the present case, in permitting to the term that extension of its original connotation which has come about through a fuller understanding of the part played by the allantoic mesoderm in the vascularisation of the placenta.

embryo as a club-shaped structure, the distal half of which is vesicular in character, it projects into the extra-embryonal coelom towards the base of the ectoplacental cone and on about the eighth day reaches the concavity of the lamina and fuses with the chorionic mesoderm which underlies the latter. Thereafter the allantoic mesoderm expands to fill the entire concavity and gradually more and more of the nutriment is conveyed to the embryo by the allantoic vessels. Meanwhile the ectoplacental trophoblast increases in thickness, and its lacunae become more numerous and complicated. Into this mass, in which the circulation of maternal blood is now established, the vascular chorio-allantoic mesoderm projects at



Fig. 15. 77—Section through the placental labyrinth of the mouse during the second half of pregnancy, showing the composition of the wall of the trophoblastic tubule. $\times 130$.

intervals and breaks it up into segments. In this way the trophoblastic lamellae are established. Jenkinson (1902) states that the chorionic trophoblast is not merely invaginated into the trophoblast of the Träger by the upgrowth of the chorio-allantoic mesoderm, but that it takes an active part in the process of ingrowth.

In these early stages of placental formation (*période de remaniement de l'ectoplacenta* of Duval), it is therefore possible (Fig. 15. 76) to distinguish the following regions : (1) The decidua basalis nearest the mesometrium consisting of typical decidual cells penetrated by enlarged maternal blood sinuses ; (2) "The penetration zone" ("Umlagerungszone," "Durchdringungszone" of German authors) which is formed by the conversion of the superficial layer of the trophoblast into giant cells and in which is found disintegrating maternal and foetal tissue as

well as extravasated maternal blood ; (3) a spongy zone of cytotrophoblast which has not yet been invaded by the foetal vessels ; (4) A zone of syncytial trophoblast honeycombed with maternal lacunae, and into which the vascular allantoic villi have penetrated ; (5) A layer of allantoic mesoderm.

The part of the placenta vascularised by the foetal vessels forms the placental labyrinth. In this region the mesodermal villi carrying allantoic capillaries soon elongate and branch, mostly parallel to one another and perpendicular to the surface of the placenta (Fig. 15. 76). At the same time the trophoblast in contact with, or adjacent to, the allantoic villi becomes syncytial. Henceforth, through later development, additional syncytium continues to be formed from the plates of cytotrophoblast capping the tips of the villi. Thus the formation of lamellae by the ingrowth of mesoderm and the differentiation of syncytiotrophoblast from the

antecedent cytotrophoblast, as well as the maximal production of decidual cells appear to be related in time and to proceed apace. Eventually, however, a stage is reached, when the cytotrophoblastic germinal tissue disappears altogether, leaving only a fine syncytial net-work, in the relatively wide meshes of which maternal blood flows (*période d'achèvement de l'ectoplacenta* of Duval), so that only foetal endothelium and very thin plates of syncytial cytoplasm separate the two blood streams, a haemochorial condition (Fig. 15. 77). The glands take no part in the formation of the placenta. Their ducts do not even act as guides to the advancing edge of the trophoblast, as in the rabbit. They are completely displaced by the rapid formation of decidual tissue and their remnants are absorbed by the giant cells.

The subsequent changes are all in the way of producing an increased surface of contact with maternal blood and lessening the thickness of tissue between it and the foetal circulation. Thus, in the definitive labyrinth the thinness of the separating membrane is such as to suggest strongly the complete disappearance in many places of the trophoblast, producing thereby a haemoendothelial relationship.

Yolk-sac placentation.

Three functional subdivisions of the yolk-sac placenta will be considered: (1) The bilaminar omphalopleur, that is, the parietal trophoblast and the distal endoderm, both of which essentially disappear early; (2) Reichert's membrane (which remains as long as there is a capsularis) and the central, annular zone of vascular giant cell tissue which is in contact with it; and (3) the inverted visceral wall of the yolk-sac (vascular splanchnopleur). No chorio-vitelline placenta is formed.

In the mouse the bilaminar omphalopleur is completely formed by the seventh day and the distal endoderm cells form a uniform though not quite continuous layer over the inner surface of the parietal ectoderm. Between the two cell layers Reichert's membrane appears. This non-cellular homogeneous collagenous membrane, is, in all probability, derived from the trophoblast and is characterised by an intense affinity for acid dyes, strong parallel birefringence, bluish white fluorescence and lack of affinity for silver (Wislocki, Deane and Dempsey, 1946). Laterally and antimesometrially the outer surface of Reichert's membrane is



Fig. 15. 78—A portion of the yolk-sac wall of the mouse before the disappearance of Reichert's membrane. On the left are the vascular villi of the splanchnopleur. 110.

associated with the giant cells of the capsularis and is bathed by circulating maternal blood (Fig. 15. 78), thus allowing substances to diffuse into the yolk sac directly (Everett, 1935). In the later stages, Reichert's membrane clothes the surface of the chorio-allantoic placenta near its junction with the "central zone" (see Fig. 15. 75).

The vascularised portion of the yolk-sac is an area of rapid growth and undergoes important changes during gestation. Beginning as a smooth simple membrane, it soon becomes specialised by development of numerous branching villi which project into the yolk-sac cavity, thereby increasing greatly the absorptive surface



Fig. 15. 79—Portion of the labyrinth near the surface of the rat's placenta, showing "endodermal sinus." $\times 200$.

of the yolk-sac placenta (Fig. 15. 78). These villi are folds of endoderm containing a vascular core and attain their maximum size around the placental disc. Anti-mesometrially the endoderm has the form of a simple layer of columnar cells in close contact with Reichert's membrane. Fig. 15. 79 shows a portion of the labyrinth near the surface of the rat's placenta and conveys an excellent idea of the so-called "endodermal sinuses" of Duval. These endodermal diverticula accompany the larger foetal blood-vessels into the superficial layers of the placenta and are believed to play an important rôle in the absorption of nutrient materials.

It has been shown by Gerard (1925) and Everett (1935) that vital dyes and other substances reach the inverted layer of the yolk sac and are absorbed by it, both before and after the disappearance of Reichert's membrane. Everett made the further interesting observation that on the twelfth day of development in the rat, toluidin blue is

completely reduced to the leuco base on entering the yolk-sac placenta. In the early period (that is before the disappearance of Reichert's membrane) the yolk-sac cavity remains shut off from the maternal tissues by Reichert's membrane, and any substances reaching the endodermal cells from maternal sources must pass through this membrane and its affiliated structures. Following the rupture of Reichert's membrane, however, the endodermal cells of the yolk-sac are exposed directly to the contents of the uterine lumen; here much debris, from the disintegrating tissues of the capsularis as well as from blood extravasations, accumulates and this doubtless provides a supplementary source of nutriment for the vitelline placenta.

The cytology of the yolk-sac epithelium of the mouse and other Rodents has recently been re-examined by Everett (1935), and Wislocki, Deane and Dempsey (1946) and there is information to be had about the Golgi apparatus, cytoplasmic

granules, and other inclusions, in the work of Jenkinson (1902), Sabotta (1903, 1911), Asai (1914), Lehner (1914), Branca (1923), Gerard (1925) and Litwer (1928). Various opinions have been expressed regarding the function of the cytoplasmic granules. They were generally looked upon by the earlier workers (Asai and others) as secretory granules which were destined to be discharged into the cavity of the yolk sac or into the uterine lumen. More recently, however, the tendency has been to regard them rather as products of absorption from the uterine fluid and variously saturated with iron and phosphatase as well as vital dyes if the latter be present. All this work and a large further body of evidence will be found summarised in the papers of Everett and of Wislocki *et al.* cited above.

The course of the blood flow through the labyrinth.

Jenkinson (1913) has studied the distribution of blood vessels in the placenta of the mouse. On the foetal surface large lacunae are filled with maternal blood brought by arterial channels passing directly through the centre of the placenta. From these lacunae, maternal blood passes through the smaller trophoblastic tubules round the foetal capillaries, and so into the efferent, maternal vessels which leave the placenta peripherally. The foetal blood vessels dip down into the labyrinth at various places surrounded by small amounts of allantoic mesoderm. They pursue a relatively straight course toward the base of the labyrinth, but unlike the maternal vessels never penetrate the whole thickness of the trophoblast. Near the base of the labyrinth the allantoic capillaries turn and pass towards the foetal surface again, but as very tortuous vessels, the endothelium of which appears to be exposed, in many areas, directly to circulating maternal blood.

If we except certain casual references by earlier writers (Duval, 1891; Chipman, 1903; Lochhead, 1922) the first definite suggestion of an "haemoendothelial" membrane came from Mossman in 1926, as a result of a study of the rabbit placenta, though he did not fully commit himself to this conclusion in respect to the mouse, rat and guinea-pig until several years later (Mossman, 1937). Since then the idea has been accepted as a matter of course by most subsequent observers. However, there have not been lacking those who remain convinced advocates of the earlier conclusions of Grosser (1909) that the thinness of the placental membrane never advances beyond the haemochorial condition. For example, Hard (1946), as the result of his observations that alkaline phosphatase tends to show a predilection for trophoblastic syncytium and that in the guinea-pig's placenta the enzyme occurs as a continuous layer throughout the labyrinth, concluded that a syncytial membrane persists as a lining for the trophoblastic tubules until term, and consequently the placenta in this species ought to be regarded as haemochorial rather than haemoendothelial. On the other hand, it should be pointed out that similar studies by Wislocki and Dempsey (1946b) and Wislocki, Deane and Dempsey (1946) in the pig and in rodents yielded results which were indecisive as to whether the rodent's placenta becomes partly or mainly haemoendothelial at term. Wislocki, Deane and Dempsey suggest "that the enzyme may have a predilection for the first cellular barrier intervening between the maternal and foetal circulations, rather than a fixed relationship to or affinity for trophoblastic syncytium." Consequently, they maintain that its occurrence as a continuous layer in the labyrinth would not necessarily be in conflict with the idea of a

haemoendothelial membrane. They state that in a haemoendothelial labyrinth the endothelium would become the limiting membrane between the maternal and foetal circulations, and in that event the endothelium might become the site of concentration of phosphatase precisely as the syncytium had been in preceding stages. Accordingly, the presence of phosphatase would not be the deciding factor as to whether the placental barrier consisted of syncytium or endothelium. However, they add, "the fact that the cytoplasm between the maternal and foetal blood channels becomes excessively thin in many places and the collagenous fibres disappear completely makes the hypothesis of a haemoendothelial barrier attractive." Nevertheless in the present state of our knowledge such a picture, while highly suggestive, must be accepted with caution.

In conclusion, it must be emphasised that these statements are not intended to imply that the accumulation of the enzyme is a negligible element in the development of the placental barrier. On the contrary, the functional efficiency of the placenta appears to be dependent on it. As Wislocki, Deane and Dempsey put it: "At the histological level it is observed that the thinnest membranous areas, regardless of what their morphological origin may have been, are plentifully provided with phosphatase, thus providing a functionally active barrier between the maternal and foetal blood streams. Moreover, although the placenta may conceivably have been rendered morphologically simpler by a reduction to a haemoendothelial state, it has not necessarily become functionally simplified so far as the important offices performed by phosphatases are concerned."

Placentation in the Guinea-pig

In the guinea-pig implantation is interstitial, and the blastocyst by its own inherent activity, embeds itself in the uterine mucosa by penetrating directly through the epithelium, its lower or abembryonic pole constituting the "implantation pole" (Von Spee, 1901; Sansom and Hill, 1931). Reichert (1862) was the first to notice that the ovum lay in a special cavity, "a little nest." (*See also* Bischoff, 1853.)

Implantation.

The fertilised ovum reaches the uterus between the third and fourth days as a morula or early blastocyst, surrounded by the zona pellucida (Von Spee, 1901; Maclaren, 1926; Squier, 1932). On the sixth day the zona disappears and embedding begins (Von Spee, 1901; Sansom and Hill, 1931), but even before this, according to Von Spee, the ovum is fixed by processes which extend from enlarged cells at the implantation pole through the zona and come into direct metabolic relationship with the uterine epithelial cells. The acquisition by the ectoplacental trophoblast of an accessory attachment to the opposite wall may delay implantation (Sansom and Hill, 1931); this so-called double implantation was first described by Von Spee. As in the mouse, the blastocyst remains small, about one-tenth of a millimetre in diameter.

At the point of contact with the mucosa, the epithelium is rapidly eroded, and absorbed along with its fat globules by the foetal ectoderm. Doubtless it undergoes extensive cytolysis before being absorbed, as postulated by Von Spee (1901)

and Sansom and Hill (1931), instead of being directly phagocytosed in the form of gross particles.

During implantation the blastocyst becomes tubular, with its long axis perpendicular to the long axis of the uterus. It exhibits, as in the mouse, an inversion of the germ-layers " or " entypy," but in the guinea-pig the amnio-embryonic vesicle is closed from the first and is separated from the Träger¹ by a space—the interamniotic (Selenka) or proexocoelomic cavity (Pytler and Strasser). The endoderm elongates in the form of a test-tube, its upper margin acquiring a temporary, purely secondary connection with the ectoplacental trophoblast just below the junction of the latter with the parietal trophoblast. The parietal trophoblast is never lined by endoderm, hence only that part of the endoderm which in the mouse has been designated as the visceral endoderm is here present.

By the eighth day the parietal trophoblast no longer exists as a continuous layer, its disruption being coincident with the growth of the endoderm to form the elongated egg-cylinder and the appearance of a new cavity in the decidual tissue—the decidual cavity. During the penetration of the epithelium by the trophoblast, some of the superficial connective tissue cells enlarge to form decidual cells. Their nuclei stain more deeply, and the protoplasm of adjacent cells fuses to form a symplasma. The degenerated tissue in its immediate neighbourhood is absorbed by the ectoderm, and the blastocyst thus comes to lie in the substance of the mucosa. According to Von Spee, the destruction of uterine tissue is effected entirely by a biochemical process; there is no evidence of absorption of formed elements by phagocytosis. (See below.)

In their description of the implantation of the guinea-pig blastocyst, Sansom and Hill state that the decidual cavity appears on the seventh day as a cleft in the anti-mesometrial portion of the symplasmic tissue immediately outside the degenerating parietal trophoblast, and as the disruption of the latter is already far advanced, the new cavity forms the direct distal continuation of the blastocyst cavity. Thus the endoderm comes to form the outer wall of the embryonal formation and is directly bathed by the nutritive fluid filling the decidual cavity. In view of these considerations, Sansom and Hill's contention seems quite logical that the endoderm of the " egg-cylinder," here as in the Muridae, plays an all important rôle in these early stages in the absorption of nutrient materials of value to the embryo.

Round the periphery of the implanted blastocyst lies the necrotic zone composed of maternal symplasma, trophoblast cells, tissue debris and blood sinuses. Later the symplasma degenerates further, the nuclei shrink and lose their chromatin, and the protoplasm becomes fibrillated and granular. Vacuolations appear in the mass, and coalesce to form a space round the ovum filled with clear fluid. In this way the implantation cavity is gradually enlarged at the expense of the capsularis. Outside it the decidual cells around the vessels survive, whilst the rest are transformed to a symplasma and absorbed. Hence the wall is sinuous. The dips are, however, filled up in part by newly formed tissue resembling granulation tissue. It encapsules the necrotic zone, and may be looked on, as in the rabbit, as a defence against the advancing ectoderm. Sansom and Hill (1931) state, however, that once it has produced the gap in the uterine epithelium through which the

¹ In the guinea-pig the amnio-embryonic mass separates from the Träger before a cavity appears in either.

blastocyst implants itself, the parietal trophoblast normally exhibits no further evidence of destructive activity, the hypertrophic growth of its cytoplasm and the great increase in the number of its nuclei, they interpret as signs of retrogression and a prelude to its ultimate dissolution.

With the growth of the blastodermic vesicle, the roof of the implantation cavity projects into the lumen of the uterus, and in time obliterates it by coming in contact and fusing, at the tenth day, with the mesometrial mucosa. Here also the cellular tissue has developed at the expense of the glands, and the surface epithelium disappears. At the fifteenth day a new lumen reappears antimesometrially, and the tissues between it and the upper wall of the yolk sac rapidly thin and finally disintegrate, thereby freely exposing the yolk sac to the uterine lumen precisely as it did in the mouse, except that here the lower wall of the yolk sac has never been present. At the same time the vessels which penetrate the necrotic zone are opened, and blood is effused into the implantation cavity.

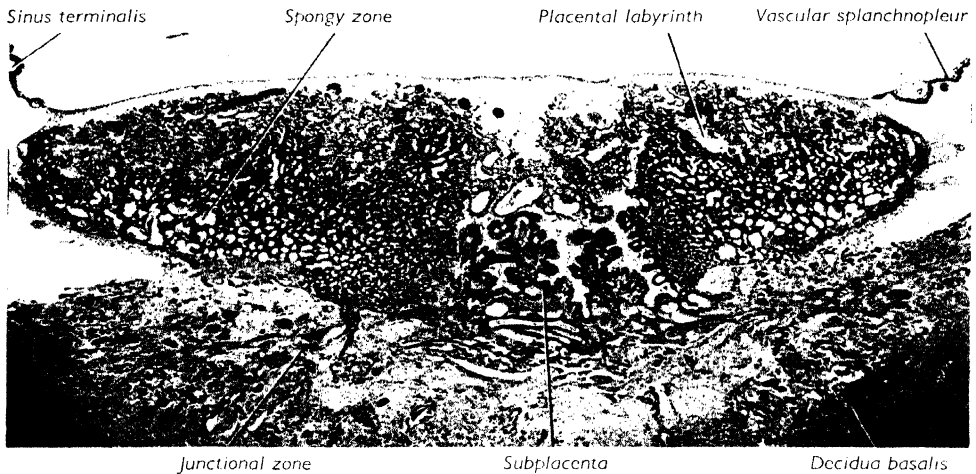


Fig. 15. 80—Section through the placenta and endometrium of a guinea-pig 23 days pregnant. . 20.

Chorio-allantoic placentation.

The chorio-allantoic placenta develops, as in the mouse, mesometrially and may be divided into four regions. These are : (1) the trophoblastic area consisting of the labyrinthine and spongy zones (Mossman, 1937) ; (2) the accessory or subplacenta (Minot, 1889) ; (3) the junctional zone ; and (4) the decidua basalis.

Towards the end of the thirteenth day the anlage of the allantois appears (Harman and Prickett 1932). It consists of a tubular passage in the body wall and a solid extra-embryonic stalk of mesoderm (Keibel, 1893). It projects into the coelom and by the end of the sixteenth day is almost as large as the embryo itself. By the twentieth day (17th day, Duval), it becomes applied to the mesoderm underlying the thickened part of the placental trophoblast, in the spaces of which circulation of maternal blood is established. The trophoblast continues to attack and absorb maternal tissue and blood, and to advance more

deeply into the decidua, while at the same time it is penetrated on the embryonic side by outgrowths of mesoderm containing branches of the allantoic vessels. In this way a complex labyrinth is formed consisting of two sets of closely paralleled

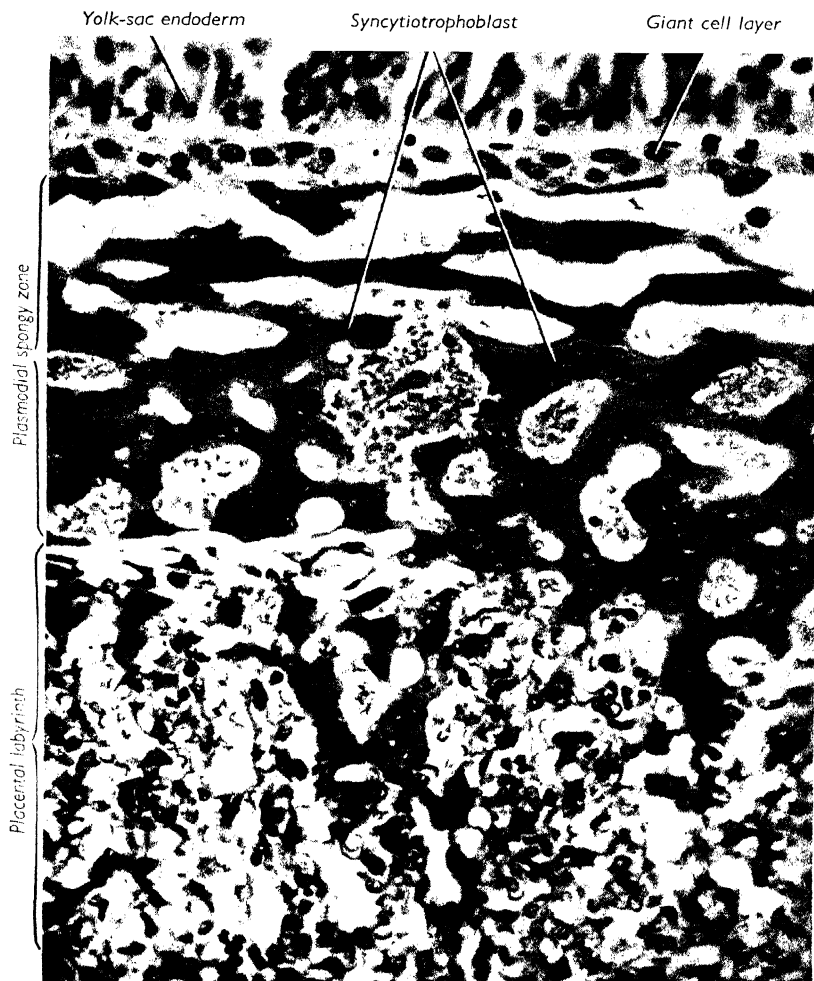


Fig. 15. 81—Margin of the guinea-pig's placenta (near term), including a portion of a border cotyledon and the peripheral spongy zone surmounted by a layer of pseudo-stratified epithelial cells of the yolk sac. $\times 200$.

tubes (1) the trophoblastic tubules, composed of fine-meshed syncytial trophoblast enclosing maternal blood, and (2) the allantoic capillaries ensheathed in delicate and discontinuous foetal mesenchyme.

One of the characteristics of the guinea-pig placenta is the peculiar manner in which the vascularised allantoic mesoderm subdivides the labyrinth, involving

(1) the formation of the accessory or subplacenta (Fig. 15. 80), (2) cotyledons, and (3) the persistence of a spongy or interlobular zone of coarse syncytial trophoblast which intervenes between the placental cotyledons and through which only maternal blood circulates. Another pertains to the relationship of the yolk-sac endoderm to the surface of the placenta. The yolk sac in this species, unlike that of some other rodents, is widely attached to the surface of the placental disc, and where this occurs its epithelium appears irregularly tufted and pseudo-stratified. Glycogen is said to be abundantly present in these endodermal cells.

These relations of the foetal tissues in the ripe placenta are shown in detail in Fig. 15. 81. It will be noted that a layer of yolk-sac endoderm lies characteristically at the surface of a giant cell layer adjoining the plasmodial spongy zone, through which maternal blood flows to and from the labyrinth below. According to Grosser (1927) the giant cells are the result of a peculiar transformation of the peripheral syncytial layer. In the labyrinth the tissues intervening between the maternal and foetal blood streams are entirely foetal; they gradually thin with the progress of gestation and the continued branching of mesodermal villi, but whether the ultimate thinnest membranes are solely endothelial thus reducing the barrier to a haemoendothelial one, or consist of syncytium and endothelium indistinguishably fused are questions which only future research will clarify (*see p. 269*).

The subplacenta is a structure of considerable size lying at the bottom of the central excavation of the placental disc (Fig. 15. 80). Unlike the spongy zone, which it adjoins on its foetal surface, its cells are closely packed in ovoid groups with few blood sinuses. On its uterine aspect it is separated from the muscularis by the tissues of the junctional zone. Practically nothing can yet be said concerning its origin and functional significance.

The complex junctional zone is composed largely of necrotic tissue derived for the most part from the decidual tissue.

Yolk-sac placentation.

The yolk sac of the guinea-pig is peculiar in that its lower wall is never developed; its cavity being lined in the area of the placental pole with ectoplacental trophoblast instead of endoderm (Harman and Prickett, 1932). The yolk-sac mesoderm develops blood islands and later becomes highly vascularised. Especially prominent is the sinus terminalis.

As in other rodents with inversion, the vascular splanchnopleur plays an important rôle in the nutrition of the embryo. Consequent on the growth of the embryo and the disappearance of the capsularis, the yolk-sac cavity early comes into open continuity with the uterine lumen and the yolk-sac splanchnopleur increases markedly in extent. At the same time folds or villi are developed in that portion of the splanchnopleur which is adjacent to the placental disc. As in the mouse, these villous folds attain their maximum development over the margins of the placental disc, whereas on the surface of the placental face the endoderm has the form of a smooth membrane of pseudo-stratified cells. The yolk sac nowhere forms a villous attachment with the placenta and endodermal sinuses are not developed.

The Histochemical Reactions of the Rodent's Placenta

The histochemistry of the rodent's placenta has received a good deal of attention, but the largest contributions on this subject are those of Wislocki and Dempsey (1945), Wislocki, Deane and Dempsey (1946), Dempsey and Wislocki (1945), Hard (1946), Dempsey and Wislocki (1947), Pritchard (1947). In these papers will be found a good summary of the literature.

In the mouse the decidual cells contain glycogen (Driessen, 1907, 1908; Jenkinson, 1913; Wislocki, Deane and Dempsey, 1946). According to Driessen, its distribution in the placenta of the white mouse before mid-term is the same as in the rabbit. It is in great abundance in the decidual cells, especially in the boundary layer (junctional zone) between the maternal and the foetal tissues. Dense concentrations also occur in the epithelium of the villous portion of the yolk sac and glycogen is also present in the secretions of the uterine lumen (Wislocki, Deane and Dempsey, 1946). No glycogen is found in the maternal endothelium. In the rat and guinea-pig, glycogen is similarly plentiful in both yolk sac and maternal placenta (*see also* Krehbiel, 1937; Pritchard, 1947).

Regarding the distribution of glycogen in the foetal placenta there is not a unanimity of opinion. Its presence in the trophoblast of the mouse (Driessen, 1908), rat (Wislocki, Deane and Dempsey, 1946) and guinea-pig (Hard, Reynolds and Winbury, 1944) is generally denied. On the other hand, Goldmann (rat, 1912) and Jenkinson (mouse, 1913) have recorded its occurrence in the foetal syncytium, while Wislocki, Deane and Dempsey (1946) show glycogen as abundantly present in the mononuclear trophoblastic giant cells in the placenta of the 12 day-old rat stained by Mitchell and Wislocki's silver method. Jenkinson states that in the mouse, glycogen makes its appearance in the trophoblast which is most directly in contact with the maternal blood, i.e. the part not penetrated by the allantoic capillaries. It lies in oblong ectodermal cells with indistinct cell-boundaries, which gradually encroach on and occupy the space previously occupied by the maternal glycogenic cells down to the muscularis. Here the glycogen remains till the end of gestation. "The trophoblastic is much more voluminous than the maternal glycogenic tissue ever was."

In the mouse, rat and guinea-pig, the epithelial cells of the villous portion of the yolk sac also contain deposits of fat (Kolster, 1903; Jenkinson, 1902; Sabotta, 1903; Wislocki, Deane and Dempsey, 1946), iron (Wislocki and Dempsey, 1945) and under experimental conditions, vital dyes (Wislocki, 1920; Everett, 1935). Fat and iron are also present in the syncytium of the placental labyrinth (Wislocki, Deane and Dempsey, 1946; Goldmann, 1912), and according to Kolster, a considerable amount of fat appears in the decidua, in which the connective tissue and endothelial cells undergo a fatty degeneration in the proximity of the giant cells.

In rodents, alkaline phosphatase is encountered in abundance in both the foetal and maternal parts of the placenta. In the mouse and rat (Wislocki, Deane and Dempsey, 1946) and in the guinea-pig (Hard, 1946), for example, the yolk-sac endoderm, the fine-meshed syncytium of the labyrinthine zone, the junctional zone, and all decidual regions exhibit a strongly positive reaction for phosphatase. It is extremely abundant in the mononuclear giant cells at the base and margin of the foetal placenta. It is said to be present in the cytotrophoblast of the mouse

(Wislocki, Deane and Dempsey, 1946), but absent in that of the guinea-pig (Hard, 1946). It is also absent from the coarse syncytium of the spongy or interlobular zone which intervenes conspicuously in the guinea-pig between the placental lobes (cotyledons), as well as in the subplacenta. Hard (1946), and Wislocki, Deane and Dempsey (1946) also report for the placenta of the guinea-pig, and of the mouse and rat respectively, an abundance of calcium in the complex junctional zone, as well as in other areas of degeneration (e.g. decidual cells) and the latter authors have associated its occurrence with the presence of glycogen and phosphatase. A useful summary of the distribution of phosphatase in the mammalian placenta will be found in the papers of Hard (1945), Dempsey and Wislocki (1947) and Pritchard (1947).

Finally the tinctorial reactions of the syncytial trophoblast deserve to be specially mentioned as there is increasing evidence that the cytoplasmic basophilia, which characterises these elements and which is abolished by previous treatment of sections with ribonuclease, is attributable to the presence of ribonucleoproteins that are implicated in protein synthesis. The foetal and maternal elements of the placenta in the mouse, rat and guinea-pig are well provided with cytoplasmic basophilic material throughout a large part of gestation. In these species it persists to a considerable extent in the spongy zone. Fig. 15.81 illustrates the condition as it is seen in the coarse syncytium of the spongy zone at the surface of the guinea-pig placenta near its margin. Abundant cytoplasmic stores of ribonucleoproteins are also present in the yolk-sac epithelium (Wislocki, Deane and Dempsey, 1946).

XI. THE PLACENTATION OF THE PRIMATES

With this section we pass to a consideration of the haemochorial villous condition, since the Primates show all variations between the typical labyrinthine placenta and the typical villous type seen in the great apes and man. The growth and differentiation of the Primate placenta has been the subject of many investigations. Those which are relevant, but which for want of space cannot be referred to here, will be found in the papers of Grosser (1927), Wislocki (1929 ; 1932), Hill, (1932), Mossman (1937) and Wislocki and Streeter (1938).

Because of Hill's authority and because his monograph will always be the principal basis for further work on the Primate placenta, it will be convenient to adopt his classification. Hill distinguishes four well defined stages : (1) a basal " Lemuroid " stage ; (2) a transitional " Tarsioid " stage ; (3) an annectant or " Pithecoid " stage, comprising the new-world and old-world monkeys ; (4) a terminal " Anthro-poid " stage, comprising the great apes and man. The characteristics implied by this classification have been reviewed in detail by Professor Hill, consequently only certain of its more general features will be considered at this point.

The Lemuroid and Tarsioid Stages.

In the *Lemuroidea* placentation is entirely different from that of the other subdivisions of the order. The placenta of the Lemurs is non-deciduate resembling strikingly the diffuse epitheliochorial type¹ found in ungulates such as the pig. On the other hand, the placenta of *Tarsius*, like that of the higher Primates, including man, is of the localised, deciduate type and haemochorial in structure.

¹ See, however, Gerard (1932) and also p. 185.

In *Tarsius* there is an unusual thickening of the trophoblast before the penetration of allantoic villi into it. According to Hill (1932) the ectoplacental trophoblast, after effecting a very localised attachment to the endometrium, is transformed completely into a mass of parenchymatous tissue, the "trophoplacental primordium."¹ With the advance of pregnancy much of this mass becomes slowly converted into multinucleated units, the cell territories of which gradually fuse together to form a network of syncytial trophoblast, which is extraordinarily massive and localised in character. Into this syncytial lamina mesodermal outgrowths of the chorion carrying foetal blood vessels are prolonged and almost immediately lacunar spaces become evident between the larger syncytial masses. Later maternal blood, at first in small quantity, begins to penetrate into these spaces. In this way a labyrinth is formed consisting of a more or less uniform, but coarse syncytial reticulum which is supported on a mesodermal framework of villous or trabecular character. The mesodermal villi are initially quite thick, but as the placenta matures they become narrower and longer, and branching repeatedly ramify throughout the greater part of the syncytiotrophoblastic network. Between the trabeculae are the irregular cleft-like lacunae in which the maternal blood circulates, whilst in their mesodermal axes, immediately below the enveloping trophoblast, are situated the branches of the umbilical arteries.

Regarding the alterations in the endometrium which accompany the development of the placenta of *Tarsius*, we refer the reader to the investigations of Hill (1932). It is apparent from his account that the maternal placenta has peculiar features in its development distinguishing it from that of both platyrrhine and catarrhine monkeys. The principal peculiarity is the extensive and long-sustained proliferation of the epithelium of the necks of the uterine glands. The interglandular connective tissue also proliferates actively, forming coarse strands of compactly arranged cells. Ultimately, both the glandular and stromal proliferations undergo degeneration, although part of the connective tissue first differentiates into decidual elements which occur grouped around the blood vessels as well as in the form of irregular masses. Concerning the function of the altered maternal tissues at the implantation site, Hill suggests that the degenerate glands and the decidual cells collectively contribute an important source of histotrophic material which is available for the nutrition of the embryo at a time prior to the complete establishment of the placental circulation. He believes, moreover, "that the curious transformation of the mesothelial covering of the yolk sac into enlarged, granular, columnar cells may be an adaptive specialisation, distinctive of the *Tarsius* yolk sac, the object of which is to facilitate the absorption of the abundant embryotrophic material present in the exo-coelom."

As concerns the circulation in the definitive placenta, Hill (1932) states simply that "The afferent (central) artery runs up through a mass of deeply staining tissue, composed apparently of degenerate syncytium and extravasated maternal blood, to open into a central sinus from which the maternal lacunae are supplied, and from these the blood drains into the larger superficial lacunae which are situated directly

¹ Hubrecht (1889), on the contrary, regarded this mass as maternal in origin and accordingly spoke of it as a "trophospongia"; the occurrence of a comparable epithelial plaque of proliferated maternal epithelium in the platyrrhine and catarrhine placenta would seem to support Hubrecht's hypothesis. However, since specimens illustrating the important phase of its initial formation are not available, final judgment as to its nature must be postponed.

below the investing chorionic mesenchyme and which collectively represent a kind of peripheral sinus and so flows round into the efferent veins." Thus it appears that the efferent pathways are solely marginal and on this score the configuration of the blood vessels of *Tarsius* bear a greater similarity to the arrangements encountered in man than in catarrhine or platyrrhine monkeys. (But *see* p. 289.)

The Pithecoïd Stage

The present description of the growth and differentiation of the placenta in the two existing groups of monkeys is mainly based on the investigations of Wislocki (1929, 1933b), Wislocki and Hartman (1929), Hill (1932) and Wislocki and Streeter (1938), although it also takes into account the older literature either directly or through the extensive quotations and discussions in the aforementioned papers.

Regarding the gross form of the placenta, there is sufficient evidence, a summary of which is given by Wislocki (1929), to discredit the view that the placenta of platyrrhine monkeys is invariably single and to show that the double discoidal type is of common occurrence in both Platyrrhines and Catarrhines. In the new-world and old-world monkeys implantation is superficial or central and there is no *decidua capsularis* (Heuser and Streeter, 1941). Moreover, in keeping with the bi-discoidal condition, there are two areas of attachment to the endometrium, a primary placenta situated either dorsally or ventrally, and a secondary placenta attached to the opposite wall; the primary attachment of blastocyst to the uterine wall being always effected by the trophoblast over the embryonal hemisphere.

The placentation of the Platyrrhina.

In the new-world monkeys the character and mode of growth of the trophoblast is perhaps the most striking feature which differentiates the placenta from that of the old-world forms. This difference in mode of growth of the trophoblast in platyrrhine monkeys arises early, the ectoplacental trophoblast (in contrast to the Catarrhines), exhibiting from the first a relatively broad attachment to the endometrium which rapidly expands with the growth of the blastocyst and uterus. This initial growth of the blastocyst is also associated with the relatively early differentiation of the primary trophoblast into a basal, cellular layer, the cytotrophoblast and a superficial, syncytial layer, the syncytiotrophoblast. The latter soon begins to penetrate the maternal, endometrial tissues, resulting at a slightly later stage in a lamina of syncytial trophoblast which is extraordinarily profuse and reticulated in character. In the meshes of this syncytial matrix a multitude of lacunar spaces arise, some of which become filled with maternal blood whilst others, more definitely outlined than the lacunae, represent enlarged maternal capillaries which have been surrounded and enclosed by the syncytiotrophoblast in its growth into the endometrium. Such intra-syncytial or intra-placental maternal capillaries, as they have been called (Hill, 1932), are not destroyed, but like the lacunar trophoblast persist into quite late stages, giving a very distinctive character to the placenta throughout a large part of its existence.

Simultaneously with the formation of the lacunae, mesodermal villi with a covering of cytotrophoblast begin to penetrate into the thick bed of syncytial trophoblast. These chorionic villi (Hill, 1932), sooner or later branch and subdivide and some few of them form direct anastomoses with corresponding branches

of other villi whilst others terminate in the syncytium. Most of the main villous stems extend, however, through the greater part of the thickness of the intervillous syncytium and some indeed actually reach the pars compacta of the decidua basalis, to which they become attached by their cytotrophoblast, though cell columns (*see* p. 287) comparable with those of the early villi of the catarrhine and anthropoid placentae are not developed.¹ In the absence of cell columns a cytotrophoblastic shell is wanting, its place being taken by irregular coarse anastomotic processes of the peripheral syncytium² which intermingle with degenerating, maternal, decidual tissue in the junctional or penetration zone.³

During the second half of pregnancy the mesoderm progresses steadily in its growth into more and more of the syncytium, the cytotrophoblast having largely



Fig. 15. 82. Portions of the villous system from the placenta of *Chrysotrrix nigrivittatus*. $\times 200$. (a) The villous branches are connected by intervillous syncytium. (b) The villi mostly lie free in the intervillous blood-space.

disappeared, being converted into villous syncytium. At the same time the intervillous syncytium becomes greatly reduced by degeneration and conversion into so-called "fibrinoid," so that ultimately more and more free mesodermal villi are formed, although over large areas of the placenta they are connected together by syncytial strands so as to form a trabecular network of quite irregular character (Fig. 15. 82). The villi now appear as relatively thin, irregularly branched strands invested by a quite thin layer of syncytium and provided with an axis of fine, fibrillar connective tissue in which are abundant capillaries, often situated immediately below the syncytial covering. Thus, in the end, the platyrrhine placenta

¹ In platyrrhine monkeys there are no anchoring villi but instead a profusion of small trabecular attachments to the decidua basalis.

² The name given to that portion of the intervillous syncytium which is related to the compacta (Hill).

³ The composite zone which results from the penetration of the processes and sprouts of the peripheral syncytium into the superficial compact zone of the decidual tissues (*see also* p. 290).

attains a condition which is not so very different from that of the catarrhine organ, although the villi "never wholly acquire the independence and freedom which characterise those of at least the majority of Catarrhines. Not only is the primary trophoblastic proliferation in the Platyrrhine immensely more massive than that of the Catarrhine, but it persists for a much longer period, with the result that the development of the Platyrrhine placenta is a slow and cumbrous process involving so much time that it only reaches a condition of what we may call structural efficiency at a quite late period of gestation and as a compensation much more use appears to be made in the earlier stages of histiotrophic materials derived from the maternal tissues than is the case in the Catarrhine" (Hill, 1932). In this connection, too, Hill's observations should be quoted to the effect "that the foetal portion of the mature Platyrrhine placenta . . . cannot adequately be described as formed simply by a trabecular network; rather it is a villous placenta in which the greatly branched and relatively slender villi are not completely free but are connected to a variable and inconstant extent by direct fusions and syncytial junctions" (Fig. 15. 82).

The maternal placenta is quite distinctive in Platyrrhines. The most obvious characteristic of this portion of the placenta is the tremendous development of an epithelial plaque of proliferated maternal epithelium. In the Platyrrhine, also, the size and degree of secretory activity of the uterine glands far surpass the related changes in the Catarrhine. The pars compacta of the decidua appears as a somewhat irregular, cellular layer, rather thin, but varying in thickness as well as in detailed histological character in the different parts of its extent. Throughout, there are present in it cells exhibiting all stages of degeneration. The syncytiotrophoblast furnishes an irregular thin layer clothing the upper surface of the pars compacta, and from it sprout-like processes penetrate into the latter and may aid in the resorption of the products of cell-degeneration. According to Wislocki (1929) the composite layer which results from the intermingling of the foetal elements (syncytiotrophoblastic sprouts) with the maternal endometrial tissues, corresponds to the composite layer which occupies a corresponding position in the catarrhine and anthropoid placenta and which Strahl (1903) has termed the *chorio-basalis*, but with this important difference that in the old-world monkeys as well as in the great apes and man, cytotrophoblast instead of syncytial trophoblast co-operates in its fusion.

As concerns the circulation of maternal blood in the mature platyrrhine placenta, Hill (1932) opines that it must be a very slow process. He has shown, moreover, that in its main structural features, that is the entry of the maternal arteries into the foetal placenta through interglandular septa, and the exit of venous blood from the base of the placenta, the circulation in platyrrhine monkeys, imitates very closely the mode of circulation encountered in typical labyrinthine placentae, details of which are to be found in Section X of this Chapter, as well as in Mossman's monograph (1937).

The placentation of the Catarrhina.

Placental development in old-world monkeys differs in certain important respects from that of the new-world forms. The trophoblast in particular becomes active at an earlier period and is endowed with more potent invasive and cytolytic properties, whilst the chorionic villi are situated not in a vascularised bed of syn-

cytotrophoblast as in Platyrrhines, but are for the most part free, and are bathed by maternal blood practically as soon as they are formed. The result is that the catarrhine placenta is capable of carrying on its full function immediately the foetal circulation is established, and it does so not only much earlier but in what would seem to be a much more efficient manner (judging from the structural relations) than is the case in the Platyrrhine (Hill, 1932). A further difference between platyrrhine and catarrhine monkeys and true of all Catarrhines thus far examined, pertains to the fact that attachment is effected when the blastocyst is smaller and less advanced than that of the Platyrrhine and that the primary trophoblastic proliferation is relatively insignificant in bulk compared with the latter. In Catarrhines, also, in keeping with the more restricted character of the trophoblastic proliferation, the area of primary attachment is smaller and more localised, whilst the endometrial reaction is correspondingly less extensive.

As concerns implantation and the very early formation of chorionic villi by the proliferation and growth of the cytotrophoblast to form cell columns, and the manner in which these columns become converted into early definitive villi by cytomorphosis of part of the cellular trophoblast into mesenchyme and angioblastic tissue as well as into syncytium, the reader is referred to the account in Chapter 14 (*also* p. 286) and to the investigations of Hertig (1935) and Wislocki and Streeter (1938). The last-named investigators, who like Hertig based their studies upon the examination of the macaque embryos in the Carnegie Embryological Collection, present a very clear and beautifully illustrated account of early and later stages, together with an interesting comparison of the catarrhine placenta with that of platyrrhine monkeys, anthropoid apes and man.

As is evident from this account, the precocious differentiation of chorionic villi which characterises the macaque blastocyst leads to extensive alterations in the configuration of the trophoblast and its associated lacunae. The early villi project into and subdivide the lacunar spaces, the walls of which appear to break down, giving rise ultimately to a continuous intervillous space, thus providing for the nourishment of the ovum by direct absorption of maternal blood. As in the lacunar spaces which it replaces, the limiting surface of the intervillous space is formed also by a thin laminar of syncytial trophoblast.

The growth and differentiation of villi in the macaque, in the manner indicated, commences on the 14th or 15th day and leads gradually to the establishment of mature relations by the 35th day of pregnancy (Wislocki and Streeter, 1938). By this time the villi have become intricately branched, stouter, longer and well vascularised; they terminate in trophoblastic cell columns (*see* p. 287) which abut against the necrotic zone of the foetal-maternal junction (Fig. 15. 83). Furthermore, the cytotrophoblast in continuity with the cell columns erupts deeply into the junctional zone and spreads out as a sheet on the surface of the decidua beneath the intervillous space. This sheet of cytotrophoblast, which is formed by the fusion of the expanded tips of the cell columns, corresponds to the so-called trophoblastic shell of human embryos (p. 288), but differs from the latter in that in the macaque, the occurrence of syncytial trophoblast in the trophoblastic shell is certainly more restricted in extent and simpler in character than is the case in the human placenta (Wislocki and Streeter, 1938). Ultimately much of the cellular trophoblast degenerates and vanishes, contributing its substance to the formation of "fibrin" which is destined to increase in extent and in thickness and to

characterise the definitive form of the junctional zone but which, nevertheless, remains less marked than in the anthropoid organ. In the opinion of Wislocki and Streeter (1938) the accumulation of fibrin in the catarrhine placenta appears to increase as the utilisation of necrotic pabulum by the trophoblastic shell diminishes, and is probably a sign that the foetal-maternal border is becoming active and stable. For further details regarding the cytology of the trophoblast in the monkey (*Macaca mulatta*) the reader should consult the elaborate account of Wislocki and Bennett (1943) and the summary on p. 293.



Fig. 15. 83—Section of Primary placenta of *Macacus rhesus* at 7 weeks. · 22.

As concerns the later differentiation of the catarrhine placenta, the form and constitution of the villi and indeed of the entire organ undergo elaboration in the direction of the anthropoid type. Both possess the same arborescent type of villus, the branches of which are attached by their apices to the decidua basalis or terminate freely in the intervillous blood-space (Fig. 15. 83). In certain Catarrhines, however, the villi in parts of the placenta are richly connected by syncytial strands (Grosser, 1927 ; Wislocki, 1929 ; Hill, 1932 and others), which so strikingly recall the villous trabecular system of the platyrrhine placenta that certain authorities have regarded them as illustrating a transitional evolutionary step of the catarrhine organ from a platyrrhine-like type. As Hill (1932), and Wislocki and

Streeter (1938) point out, however, these syncytial connections in the Catarrhine (Fig. 15. 84) arise relatively late as sprout-like outgrowths of the syncytium covering the villi and do not represent the remains of an originally profuse intervillous connection. Consequently, no evolutionary significance should be attached to them. It should be pointed out, moreover, that this manner of proliferation of the syncytium is not exclusively a character of the Catarrhines, since Hill (1932) has described and figured a similar fusion of adjacent villi in the placenta of man and the gorilla (see Fig. 15. 88). On the whole, though, it appears to be a more pronounced and widespread feature in Catarrhines (Hill).

In a later section we shall consider certain special characteristics and properties of the trophoblast in man and in the monkey, but from what has been said in the preceding paragraphs, it would appear that the peculiarities which distinguish the foetal portion of the Catarrhine placenta from that of the Anthropoids are to be

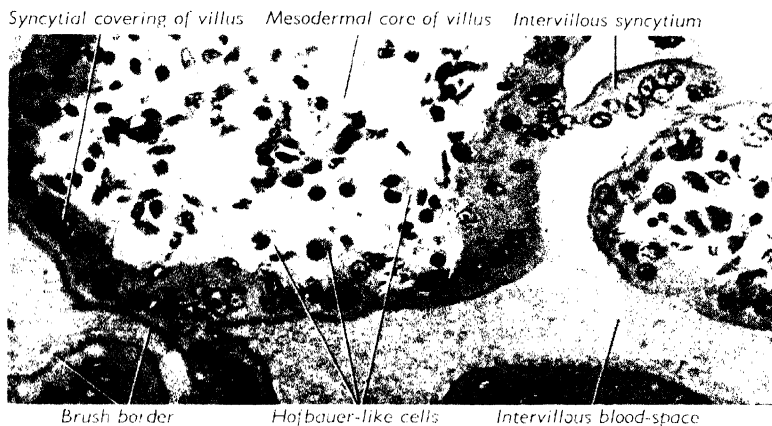


Fig. 15. 84. Detail of the placental villi shown in Fig. 15. 83. $\times 370$.

regarded as mere differences in detail or degree. The maternal placenta in the two forms, on the contrary, exhibits rather pronounced differences in early stages; the principal one being the formation in the Catarrhine of an epithelial plaque similar to, but less extensive than, that described in the platyrrhine. In later stages, however, as the epithelial plaque degenerates and is absorbed by the trophoblast, the junctional zone begins to assume a form resembling that in man. Thus in addition to the cytotrophoblast and syncytial strands which are integral parts of the trophoblastic shell, there are present in the border zone and its vicinity a layer of necrotic, maternal tissue consisting of similar elements to those encountered in man (see p. 290). In the monkey (*Macaca mulatta*), also, the boundary between the junctional zone and the compacta is relatively indistinct and well defined decidual cells are abundant in both zones. The decidual cells are, however, distinctly smaller and not nearly so extensive as those in man (Wislocki and Bennett, 1943).

For details regarding the circulation of maternal blood in the catarrhine placenta reference should be made to the account of Herberg (1935). According to this investigator, the maternal blood reaches the intervillous space through a series of

large coiled arteries which enter centrally and basally beneath the placenta, while the blood leaves the organ along its margin through the border sinus with its venous plexus, as well as basally through the floor of the intervillous space. Comparing these arrangements with the description of the circulation in the human placenta (p. 292), it will be seen that the catarrhine monkeys differ in certain particulars from man, the main one being the presence in the former of two systems of drainage—one marginal the other central—which are virtually independent of one another, whereas in man the efferent pathways are solely marginal.

Human Placentation. (The Anthropoid stage)

The chorio-allantoic placenta of this species is fundamentally different from that of the rodents. It is a deciduous, discoidal, villous placenta of the haemochorial type; or, according to Grossers' classification, it would be a *placenta conjuncta haemochorialis olliformis*.

The character and mode of growth of the trophoblast in the human has long been the subject of morphological discussion, and no serious attempt will be made to cover the literature relating to placentation in man as thoroughly as that relating to other species, nor will any extensive account be given of successive phases of placental growth and differentiation. Adequate reviews from the historical and other points of view have been made by Grosser (1910, 1927); Krölling (1927); Mayer, Vogt and Sietz (1929) and others. There are, however, a limited number of facts which should be emphasised in order to illustrate the essential steps in the development of the human placenta and to provide a basis for comparison with the development of the more primitive haemochorial labyrinthine placenta of other orders.

Implantation.

Studies and interpretations of the manner of implantation of the human blastocyst are numerous. The extensive literature may be approached by way of several excellent contributions, such as those of Teacher (1925); Streeter (1926); Grosser (1910, 1927); Hill (1932); Krafka (1941); Hertig and Rock (1941, 1945). As the outstanding characteristic of the human blastocyst, Hill describes the extraordinary precocity it exhibits as demonstrated, for example, in the relations it early acquires to the uterine lining and in the remarkably early differentiation of its trophoblast and its extra-embryonic mesoderm. Unlike that of the old-world and new-world monkeys (Wislocki, 1929, 1930; Hill, 1932; Wislocki and Streeter, 1938) the developing egg undergoes interstitial implantation, a decidua capsularis is formed, and there is an initial growth of the trophoblast over the entire surface of the blastocyst. From the outset, however, as shown by the earliest implantations known to us—those of approximately 7 and 9 days of age, described by Hertig and Rock (1945), the trophoblast over the embryonic hemisphere takes a more active part in the maturation of the blastocyst than the trophoblast which constitutes the abembryonic or luminal pole of the vesicle. This results in the immediate increase in the amount of trophoblast adjacent to the decidua basalis, and especially in the differentiation of syncytium which almost at once gives rise to intercommunicating lacunar spaces which become filled with maternal blood. This phase of trophoblastic development is sometimes described as "pre-villous."

Blood lacunae are already present in the 9-day old human ovum and represent the earliest stages in the formation of the intervillous spaces of the future placenta (Grosser, 1910 ; Wislocki and Streeter, 1938 ; Hertig and Rock, 1941). These lacunae are very generally believed to arise as intra- and inter-cellular vacuoles (Grosser, 1910 ; Streeter, 1926 ; Hertig and Rock, 1941 ; and others), although Peters (1899) supposed that they were formed by the blood streaming out under pressure from the eroded maternal vessels and excavating channels in the trophoblast. In them the blood does not clot, the syncytium acting as an endothelium, but at a certain stage the blood begins to circulate and continues to do so throughout pregnancy. The maternal blood within the trophoblastic lacunae is at first small in amount, and there is reason to believe that the lacunar circulation is comparatively sluggish, but exact information concerning the rate of flow is lacking.

The vascular relations in the implantation site are not surely known ; but a fairly constant picture in the majority of early implantations is the presence of basal sinusoids. The origin of these sinusoids appears to be from the terminal small branches of the spiral arterioles, that is to say, they are the hypertrophied and dilated arterio-venous anastomoses of the endometrium in the region of the implanted ovum (Hertig and Rock, 1941). In all instances these sinusoids drain by small openings into the trophoblastic lacunae and there can be little doubt that one of the benefits of sinus formation is the lowering of blood pressure to prevent mechanical abortion (Ramsey, 1938). Also, the reduced flow might be favourable for some of the activities of the syncytial layer, e.g. phagocytosis or lysis of maternal blood corpuscles. Daron's (1936) studies on the spiral arteries indicate that arterial spasm, as well as the tortuosity of the vessels themselves are the effective agents in controlling the blood flow, but whether or not the coiled arteries open into the lacunae at this early stage is still a disputed point. For further details regarding this question, see Ortmann (1938) and Krafka (1941).

Three hypotheses may be considered with regard to the manner of growth and penetration of the trophoblast in the formation of the Primate placenta : (1) that there are constitutional differences in the blastocyst walls, the trophoblast of man and the anthropoid apes being endowed with more active cytolytic and invasive properties than that of the remaining members of the order (Hill, 1932), (2) that the greater invasiveness and precocity of the human blastocyst as compared with that of the monkeys, may be attributable to the changing character of the endometrium, which becomes in man less resistant to the invasive proclivities of the trophoblast, whereas in monkeys the more pronounced reactions of the endometrium may block the rapid extension of the trophoblast, leading to central instead of interstitial implantation (Wislocki and Streeter, 1938), or (3) that the ovum and the endometrium have complementary functions and that neither is " chiefly " responsible for implantation (Fawcett *et al.*, 1947). There are no specific data to indicate which of these hypotheses is correct.

Formation of chorionic villi and the trophoblastic shell.

The invasive action of the trophoblast continues actively for some time after the blastocyst has implanted itself in the vascular, subepithelial, endometrial tissue, but becomes relatively slower as the placenta matures. The trophoblast occurs partly as cytotrophoblast and partly also as syncytial trophoblast constituting the outlying and more invasive layer. Under its influence the endothelial

walls of the maternal capillaries are gradually destroyed and more and more blood escapes into the lacunar spaces formed in the syncytium.¹

The growth of the syncytium does not occur as a solid mass, but as trabeculae and cords, which give the entire chorion a villous appearance and may be termed *primitive syncytial villi*. Into the syncytium project outgrowths of the cytotrophoblast forming the *primary chorionic villi*, which thus consist wholly of epithelium with no connective tissue cores (cellular villi of Peters and Leopold). According to Bryce and Teacher (1908), the cytotrophoblast tends to grow out not so

much into the strands of syncytial trophoblast as into the spaces between them. Primary villi are already present in the early human ova of the eleventh and twelfth days (Streeter, 1926 ; Hertig and Rock, 1941 ; and others).

Early in the third week of pregnancy mesoderm begins to appear in the proximal, attached portions of the primary villi, and penetrating into the cellular buds converts these into *secondary villi*. This growth is due to the active proliferation and differentiation of the cellular trophoblast, the cells which are delaminated from its inner surface being converted into typical mesenchymal cells (Hertig, 1935 ; Streeter, 1938 ; Heuser, 1938). Simultaneously with the formation of the chorionic mesoderm, the first blood vessels arise from angioblastic cells which are also derived directly from the cellular trophoblast (Hertig). These vessels are continuous with those of the



Fig. 15. 85—A transverse section of human placental villi at 2 months, showing traditional position of cellular and syncytial trophoblast. $\times 210$.

connecting stalk and later with those of the umbilical cord. Such villi with a vascular connective tissue core and a covering epithelial layer of cellular and syncytial trophoblast are now called *tertiary* or *definitive villi* (Fig. 15. 85). The syncytial layer lies outermost and forms a relatively thin mantle of variable thickness bounding the intervillous space. Under the syncytium is the cellular or Langhans' layer ; its cells are in a state of active division (Hofbauer, 1905 ; Grosser, 1910, 1927) and it is generally regarded as the germinal zone of the outer syncytial layer.

By a similar process other villi are developed till the whole system becomes branched like a tree, the main concentration of villi being marked off by thin

¹ Open vessels may, however, be seen far removed from the syncytium, and syncytium may be seen adjacent to vessels without evidence of lysis.

placental septa into lobules known as *cotyledons*. Of these villi (Fig. 15. 86) some may hang free in the intervillous space (*free* or *absorbing villi*), while others become attached to the decidua (*anchoring villi*). At first the attached ends are syncytial, but



Fig. 15. 86—Section of human uterus and placenta at two months. x 25.

later the cytotrophoblast at their tips proliferates and forms thick rounded masses, the *cytotrophoblastic cell columns* over which much of the syncytium disappears (Fig. 15. 87). The cell columns extend from the tips of the villi and adhere firmly to the floor of the intervillous space, where they spread out and unite over

the eroded uterine surface to form the so-called *trophoblastic shell*.¹ The outer surface of the shell constitutes the boundary between the foetal and maternal parts of the placenta.

The core in the young villi consists of a matrix, homogeneous or delicately fibrillated. In it are placed the blood vessels and connective tissue cells with long branching processes, which form a network in the matrix, and probably



Fig. 15. 87—Human. Section through junction of chorion and decidua basalis in the second month, showing anchoring villi and cell columns. $\times 132$.

provide a series of lymph-channels (Bonnet, 1903b). Another type of cell conspicuously larger than the ordinary connective tissue cell also occurs in the stroma—the so-called *Hofbauer cells*. They are found in all stages of pregnancy but are more abundant during the early months. Their significance is still uncertain, but in man, they appear to be phagocytic and from the staining reaction of their granules are very generally believed to be histiocytes (Lewis, 1924). Similar cells are present in the 50-day monkey (*Macaca mulatta*) placenta and appear to be delaminated from the associated trophoblast. (See Fig. 15. 84.)

During the early weeks of pregnancy branching villi project from the entire surface of the chorion, but those in relation to the capsularis do not branch so much, and even at the end of the first month they are fewer in number than over the basalis (Kastschenko, 1885). Later, however, when the blood supply of the capsularis is reduced, the villi in relation to it degenerate, and are compressed between the chorion and the apposed decidua capsularis and vera giving rise to the *chorion laeve*.

Over the basalis, on the other hand, where they are favoured with an abundant blood supply, the villi continue to grow and becoming increasingly branched, longer and more slender, give rise to the *chorion frondosum* (placenta foetalis), which is essentially a mass of foetal villi between which maternal blood circulates. It is the conjoined chorion frondosum and decidua basalis (placenta materna) which together constitute the placenta.

¹ The so-called *cell islands or nodes* are also masses of cytotrophoblast with a variable amount of associated syncytium, which are attached to the ends of the villi, but otherwise lie free in the intervillous space. The nodes are very prone to degeneration.

Coincidentally, the maternal blood spaces between the villi expand, intercommunicate more freely than formerly, and completely surround the villous growth; the blood spaces thus become transformed into the *intervillous space* of the definitive placenta. For a time its walls are lined by trophoblast, but later some of the lining becomes replaced with fibrinoid material. The intervillous space occupies the territory between the chorionic membrane on the foetal side and the decidua basalis on the maternal side; at the periphery of the placenta it is said to form the *marginal sinus*, a villus-free channel which does not encircle the placenta completely, and may play a prominent rôle as one of the efferent channels of the main space. By the attaching villi the spongy mass is anchored to the decidual surface. The attached ends may excavate the decidua but there is no great degree of penetration. The presence of a *marginal sinus* in the human placenta has been denied.

The decidual or stromal reaction.

One of the characteristics of all early human implantations is the fact that the endometrium is in the premenstrual phase (Grosser, 1910). But once the initial penetration of the ovum has been effected the decidual or stromal reaction begins. It is characterised in the human by the almost complete absence of proliferation of the uterine epithelium, by the early occurrence of degenerative changes in the walls of the blood vessels and by the relative inactivity of the uterine glands. The vessels are dilated, and blood extravasations occur between the cells and into the uterine lumen. The tissue is oedematous and spongy, and the swollen cells often appear to be floating free in a fluid (v. Heukelom, 1898). These changes are especially marked near the ovum, and they give rise to an elevation which marks the resting place of an early blastocyst. Similar changes occur also in the general endometrium, suggesting that two processes are simultaneously active, viz., invasion of the vascular bed at the implantation site by trophoblast, and a general extravasation accompanying the oedema of an impending menstruation (Krafka, 1941). In the macaque monkey, the presence of fresh blood in some of the endometrial glands is recorded for the first time on the 17th day (Wislocki and Streeter, 1938) and continuing for varying periods of from two to four weeks, accounts for the appearance of traces of blood in the vaginal fluid. This is the so-called "placental sign" noted by Hartman (1928). According to Krafka (1941), "the blood-in-the-glands reaction is a sharply defined process, occurring in man, as in the monkey, at a time consistent with an expected menstruation which is partially held in abeyance by pregnancy. It is not due to the erosive action of the syncytium, since it does not occur during the period of most active invasion."

The mucosa is differentiated into a superficial layer, the *stratum compactum*, an intermediate *stratum spongiosum* in which are the enlarged middle portions of the uterine glands, arterioles, venules and lymphatics, and a deeper *stratum basale* in which there is a marked reduction in the gland size, in both lumen and epithelial height. In the *stratum compactum*, the connective tissue cells undergo active division, and they enlarge, owing to storage of glycogen and lipoids in their cytoplasm, to form characteristic, lightly staining, polyhedral *decidual cells*. Before the excavation of the implantation cavity they are probably not found, though Peters (1899) described the commencement of a decidual change before that stage.

The capillaries dilate to sinuses, and new vessels are also formed in the compacta. Many of them are opened by the trophoblast and perhaps by the giant cells and gradually more and more blood is effused into the trophoblastic lacunae. In some early ova, e.g. Merttens (1894), Hugo (Stieve, 1926), Torpin (Krafka, 1941), a second type of decidual reaction has been described, in which the cells retain the fusiform character of the normal stroma, but are greatly enlarged and lie parallel to the surface in cavities bordered by vasofactive strands. The significance of this reaction is unknown. The decidual change arises first in the connective tissue cells near the ovum and later it extends more deeply in the compacta. There is no special perivascular development as in the rabbit, though Brewer (1938) has noted a decidual-like reaction in the stroma of the vascular septa through which the spiral arteries course. Mitosis in the endothelium of the vascular plexuses have been occasionally recorded (Brewer), while the presence of swollen, endothelial cells, especially in the arteries of the junctional zone, has frequently been observed. (For a further discussion of the problem of "swollen endothelium," see Grosser, 1910; Mossman, 1937; and Wislocki and Streeter, 1938.)

The foetal-maternal junction.

Round the blastodermic vesicle is a characteristic deeply staining zone of degenerating tissue in which foetal and maternal elements intermingle; it is variously referred to in the human and other primates as the *chorio-basalis* (Strahl), *penetration* (Grosser), *transition* (Peters), *junctional* (Wislocki), *composite* (Hill), *border* (Brewer) or *detritus zone* (Bonnet). In man this composite zone is somewhat less clearly defined than in the monkey (Wislocki and Streeter, 1938), and in ordinary histological preparations it is exceedingly difficult to assign exact limits to the maternal and foetal tissues, since many of the decidual cells and the trophoblastic elements are degenerating and therefore atypical. However, the decidual edge may be readily identified with silver, owing to the absence of argyrophil reticular fibrils at the border of the trophoblastic shell where foetal tissues begin (Keibel, 1915; Wislocki and Bennett, 1943). Particularly prominent in the junctional zone are certain large, mononuclear cells (*Giant syncytial cells*; Grosser, 1910) which extend beyond the limits of the trophoblastic shell and erupt in the decidual stroma. In general their size is some four to five times that of a granular leucocyte and cell margins are distinct. These syncytial giant cells appear to be engaged in disintegrating mucosal tissue and the vascular seepage seen in the immediate neighbourhood of the cells may represent an enzymatic action upon the capillaries similar to that exerted by the chorionic epithelium (Ramsey, 1938). Their time of appearance is consistent with the time when the "cell columns" break through the "shell," (Krafka, 1941); hence it seems reasonable to suppose that they are foetal elements rather than stromal cells in an early stage of degeneration.

From the foregoing considerations, it is apparent that the junctional zone must contain degenerating maternal cells of various sorts and debris, besides trophoblastic tissue in the form of syncytium as well as in the shape of cytotrophoblast. Moreover, in the days immediately following implantation, the trophoblast and especially the syncytium, is evidently capable of engulfing and digesting the degenerating maternal cells with which it is actually in contact or which it surrounds

(Peters, 1899 ; Brewer, 1937 ; Hertig and Rock, 1941). Hence this material must be regarded as a continuing source of nutriment for the trophoblastic shell during its active phase of growth and differentiation.

Later changes in structure of uterus and placenta.

As pregnancy advances marked degenerative changes occur in the maternal and foetal parts of the placenta. The most notable change in the villi is the gradual disappearance of the cytotrophoblast, most of which becomes converted into syncytium (Fig. 15, 88). Even the cell columns (Zellsaulen) tend to disappear from the tips of the villi and their connective tissue comes in contact with the decidua. The mesenchymal core of the villus becomes progressively denser and coarser and differs materially from the mucoid tissue (Wharton's jelly) of earlier months. Fibrinous changes are frequent in the remnants of the cytotrophoblast and in the mesoderm, and occupying the former position of the Langhans' cells, a thicker or thinner layer of canalised fibrin may be observed. At the same time the syncytium clothing the villi becomes simplified with age and is reduced in thickness. Where it adjoins allantoic capillaries it often becomes so attenuated that only the thinnest "epithelial plates" and wisps of argyrophil reticular fibrils separate the foetal vessels from the blood in the intervillous space. According to Grosser (1910) the epithelium may be wanting at many places on the surface of the villi. In such cases so-called placental fibrin occurs in the villi. (See, however, Wislocki and Bennett, 1943.)

The decidua basalis, after reaching its full development during the third month is gradually thinned out. This may be partly due to the stretching of the tissue by the increasing growth of the uterine contents, but it would seem also to depend on conditions of malnutrition caused by the blood-stasis (Bonnet, 1903 b), and by the choking of the lymphatics by the decidual development (Webster, 1901). The resulting degeneration takes the form of a coagulation necrosis or symplasma, as shown by the occurrence of *Nitabuch's fibrin stria*, which are comparable to the fibrinous deposits in the rabbit's placenta. Nitabuch's stria may be seen as early as the sixth week, and even earlier in the capsularis. They gradually extend more deeply into the substance of the decidua, and also occur in the vessel walls. They are, however, most marked at or near the junction of the maternal and foetal tissues.¹ That they are due to the influence of the ovum is highly probable from their absence in the vera. For a critical discussion of the question of "Fibrinoid," the reader is referred to Grosser (1925b, 1927), and Wislocki and Bennett (1943).

According to Webster (1901), there may be a new formation of decidual cells during pregnancy, from irregularly distributed groups of active cells which are present at all periods in the maternal part of the placenta.

The uterine glands take no part in the formation of the placenta. By the sixth week their superficial parts are largely obliterated, and the deeper parts have degenerated. At a later stage, only a few blind ends are seen next to the muscular layer. Though their epithelium offers a considerable degree of resistance, and is visible for a long time, its secretory power is probably lost very early. In the vera

¹ Similar masses appear also in later stages in the wall of the intervillous space (*Rohr's stria*), and beneath the chorionic plate (*Langhans' stria*).

the glands increase in size and secrete actively for a time. Their secretion is found as a milky fluid in the uterine cavity.

The definitive anatomical condition of the circulation in the human placenta is reached by the second month. The maternal vascular supply as described by Spanner (1935) consists of a series of coiled arteries which pass obliquely through the basal plate and open by extremely narrow nozzles into the intervillous space. According to Spanner, each vessel shows three successive parts. The first portion is a short, slightly convoluted connecting piece which divides into two branches. Each branch forms an extremely wide, coiled segment, the "Reserve-schlauch"; this portion gives off a number of minute branches to the decidua

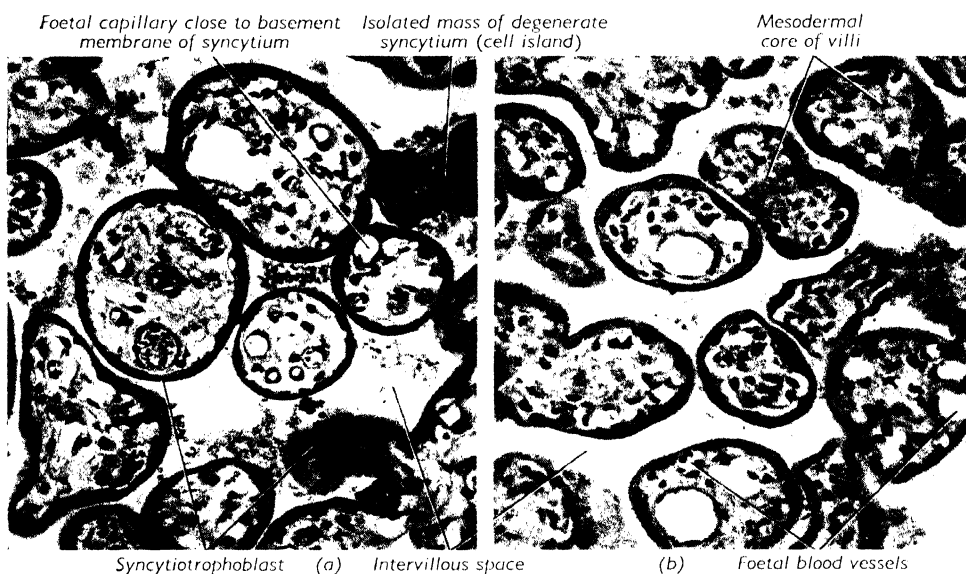


Fig. 15. 88—Comparison of chorionic villi in man and gorilla. $\times 190$.

(a) Section of human placenta at eighth month. (b) Advanced placenta of gorilla.

Placentation in man and the anthropoid apes is so very similar that in later stages the placentae are practically indistinguishable.

basalis before breaking up into a variable number of short, terminal end-pieces. These—forming the third portion or "Mündungstücke" are funnel-shaped and discharge their contents into the intervillous space by extremely narrow ostia. The maternal arterial blood which has thus entered the bases of the sinuses then flows through the placental labyrinth towards the chorionic plate. Here, the venous blood spreads horizontally in the sub-chorial portion of the intervillous space towards the marginal venous sinus, from which it is drained by a rich maternal plexus of venous tributaries situated in the neighbouring decidua. Thus all the venous outlets are limited to the outer portions of the marginal cotyledons, the central lobules lacking all venous connections. On the other hand, Ortmann (1938) reported the existence of direct venous outlets from the central cotyledons during the fourth week of pregnancy. If this be true, as is quite possible, then the conditions prevailing in the mature placenta must differ materially from those

found in the early stages of development, and accordingly, we must postulate a remarkable shift in the vascular pattern during the later stages of gestation.

In man, the contact area between the maternal and foetal circulations is very great; Christoffersen (1934) has estimated it at between 10 and 13 square metres, considerably greater than the $6\frac{1}{2}$ square metres given by Dodds (1924) and Reck (1924) and much in excess of the surface area of the skin of the new born. The capacity of the intervillous space varies greatly and at the end of pregnancy has been stated to be as high as 300 cubic centimetres or as low as 90 cubic centimetres (Jonen, 1927).

Details about the vascular supply of the chorionic villi are to be found in the papers of Shordania (1929), Spanner (1935), Kearns (1939), and by Bacsich and Smout (1938). According to Spanner, each cotyledon of the placenta is supplied by an arterial branch which enters the stem villus and extends towards the basal plate, giving off along its course a number of recurved branches which supply the subsidiary villi. In the stroma of the terminal villi the foetal vessels break up into capillaries, the walls of which have the most delicate wisps of reticular fibrils accompanying them (Wislocki and Bennett, 1943). Here the capillary plexuses displace the trophoblastic cells to some degree, and compress their cytoplasm into thinned out plates. The intimate association of foetal capillaries and syncytium is illustrated in Fig. 15. 88. From the smallest villi, blood is returned to the foetus by innumerable veins which accompany the arteries. Many of the larger venous tributaries are provided with muscular sphincters—the so-called “Throttle Veins”—and these are believed to control the returning blood flow.

Structure and functions of the epithelial investment of the villi.

The cellular layer of the villi (Langhans' layer) is a temporary structure, and disappears to a great extent early in pregnancy without signs that the cells actually degenerate in any great numbers. According to Merttens (1894), Van Cauwenbergh (1907), and Wislocki and Bennett (1943), some Langhans' cells persist until term. Mitoses are abundant in the cellular layer (Hofbauer, 1905; Grosser, 1910, 1927; Hertig and Rock, 1941; and others), and it is generally regarded as the germinal zone of the outer syncytial layer, as well as of the mesoderm and the angioblastic cells of the chorionic villi (Hertig, 1935; Wislocki and Streeter, 1938; Krafka, 1941). Processes of the cellular layer precede the mesoblastic outgrowths in the formation of the villi, and by a special proliferation of the cells of the tips of the villi—the *cytotrophoblastic cell columns*—an attachment to the decidua is effected. Grosser (1910, 1927) believes that these columns are made up of a second generation of cytotrophoblast which is developed at the time of establishment of the maternal circulation, i.e., at the beginning of the haemotrophic period. Langhans' epithelium is composed of large, discrete, pale staining cells with relatively large nuclei. The cells which compose the columns, on the other hand, vary considerably in size; those at the base approximate the *Langhans' cells*, with which they are in continuity, and like the latter have repeatedly been seen in mitosis; those in the core and tip ends are larger, paler and more spheroidal. These columns are the growing zones of the trophoblast and have disappeared for the most part by the end of the second or third month.

The cytotrophoblast of the cell-columns and of the trophoblastic shell is conspicuously vacuolated and plentifully supplied with glycogen, whereas the Langhans

cells exhibit a lesser degree of vacuolisation and the glycogen is correspondingly reduced. Glycogen is said to have completely disappeared from the human placenta by term (Driessen, 1907). No lipid droplets occur normally in the cellular trophoblast. The Langhans' cells are characterised further by an interstitial matrix which surrounds and separates them from one another and intervenes between them and the syncytial trophoblast. The source of this material—the so-called *fibrinoid* (Grosser, 1910) is still in dispute, being variously considered to be the blood fibrin, the tissue fibres, stroma cells and lastly the trophoblast. Interesting in this connection are certain observations of Wislocki and Bennett (1943). They have demonstrated by a variety of cytological procedures that this ground substance is a protein-containing fluid of mixed origin, derived mainly from the decidua and maternal blood streams but containing also substances released by the cellular trophoblast. Fibrin they believe becomes variously deposited in it.

While present the cellular layer lies in the path by which the nutriment for the foetus is carried to the capillaries of the villus, and the combined observations of several investigators indicate, that the Langhans' cells are actively metabolising and secreting cells which are taking in certain substances at the same time that they are engaged in discharging others, either cytolytic or hormonal. Friedheim (1929), Sengupta (1935), Gey, Jones and Hellman (1938) and others, have observed living cytotrophoblast in tissue cultures, and from such preparations chorionic gonadotrophin has been recovered (Jones, Gey and Gey, 1943). Furthermore, in the human and in the chimpanzee (Elder and Bruhn, 1939), there appears to be a time relationship between the curve of excretion of chorionic gonadotrophin, and the maximum expansion and decline of the cytotrophoblast, but with no other cellular components of the placenta. These observations indicate that the cytotrophoblast may be the source of the hormone. More detailed evidence is given by Wislocki and Bennett (1943).

The widespread occurrence of necrosis and other changes in the immediate vicinity of the primate ovum has long been regarded as being due to the liberation of cytolytic substances from the surface of the egg. Opinion has been divided, however, as to the source of these enzymic substances; some have regarded them as emanating principally from the cytotrophoblast (Gräfenberg, 1909; Kiss, 1921; Friedheim, 1929) whereas others have maintained that it is the syncytium rather than the cytotrophoblast which destroys the decidua (Bryce and Teacher, 1908; Hill, 1932). Unfortunately the data are insufficient to present a critical examination, but some evidence indicates that perhaps in the early human and monkey placenta, syncytial lysis and engulfment of cells in the sense in which phagocytosis is usually defined, appears to be restricted to the peripheral trophoblast in the relatively early stages of development. In later stages, on the other hand, when the syncytial trophoblast has largely disappeared from the trophoblastic shell, it is evident that the cytotrophoblast is principally responsible for the lysis and degeneration of the neighbouring decidua (Wislocki and Streeter, 1938).

In contrast to the cellular trophoblast, the syncytium is more permanent. In the 7-day old human ovum (Hertig and Rock) it already constitutes a considerable polar mass, and a similar thickening over the whole or part of the circumference of the blastocyst occurs in all the Deciduata. In the great apes (gibbon, Selenka, 1899; orang, Strahl, 1903; chimpanzee, Elder, Hartman and

Heuser, 1938 ; gorilla, Hill, 1932), and in man where a decidua capsularis exists, the early proliferation appears to be related to the excavation of the cavity in which the ovum lies, and it seems likely that the initial thickening may be induced by some substance or substances which are liberated by the endometrium.

In the later stages of pregnancy the syncytium forms an attenuated membrane over the villi, but probably never thins out to a point where it disappears leaving only the foetal endothelium, as so often happens in the higher rodents. Indeed, it seems well established that the separating membrane between the foetal and maternal streams is always composed of three layers, namely, endothelium, a delicate network of argyrophil reticular fibrils and syncytium (Wislocki and Bennett 1943). Bremer (1916), it will be recalled, likened the thinned-out epithelial plates overlying the foetal capillaries to the glomerular epithelium and regarded them as inert areas through which the foetal waste products are excreted. Nevertheless, thin as these areas may appear to be, they are cytologically very complex (Wislocki and Bennett) and the combined observations of Cunningham (1920, 1923), and of Wislocki and Bennett indicate that they must be capable of transmitting substances from the mother, as well as in the opposite direction.

The syncytial nuclei are numerous, small and aggregated in clumps, and most of the authorities agree on the absence of mitosis, some holding that they divide directly, others that they have lost the power of division. The possibility of direct division in the syncytium has been accepted by Grosser (1910), Florian, (1928), Johnston (1941) and others, but the criteria for its identification in this tissue are far from convincing. The protoplasm is foamy and finely granular, and in man and the monkey (Figs. 75, 84 and 85) it is condensed superficially to form a layer which bears a "striated" or "brush" border (Burstenbesatz). This consists as seen in fixed specimens, of a series of fine striae running perpendicularly to the surface, and its structure and function have been much discussed since it was first described by Minot (1889). Some have denied its existence during life and ascribed it wholly to the method of preparation. But Hofbauer (1905) has shown that the fresher a specimen is when obtained, the easier it is to demonstrate the striae by methods of staining, and, therefore, it is probably a vital structure. Kast-schenko (1885) looked on the striae as fine hairs which projected from the surface of the cells and by their vibrations created a stream in the maternal blood of the intervillous space. But no movements have yet been observed, and von Lenhossek calls them "Stereozilien," or stationary cilia although clearly discernible basal granules or blepharoblasts have rarely been described. A detailed description of the placental brush border in the macaque monkey and in man is given by Wislocki and Bennett (1943). In the human placenta Graf v. Spee (1896) attributes the appearance to the teasing out of the surface of the protoplasm, and looks on it as evidence of a strong flow of fluid through the syncytium.

Various other surface irregularities varying from minute vacuoles to delicate cytoplasmic streamers and fronds have also been described by Wislocki and Bennett (1943). The presence of vacuoles they interpret as a possible example of pinocytosis or water imbibition (Lewis, 1931), rather than signs of degeneration (Florian, 1928), and in this they are supported by the observations of Hertig and Edmonds (1940). On the other hand, the cytological picture of frond-like and lacy arrangements is regarded as indicative of the liberation of other substances from the surface of the trophoblast. Also, on the basis of the cytological picture, particularly

the protoplasmic streamers and strands of syncytium which penetrate the surface of the decidua or form on the surface of the villi, a certain degree of amoeboid motility is postulated (Hofbauer, 1905 ; Grosser, 1910).

The syncytial trophoblast of the human placenta is characterised further by a variety of substances (Dempsey and Wislocki, 1944 ; 1945). Acid phosphatase accumulates in the syncytium towards the very end of gestation ; it occurs principally in the nuclei and to a lesser extent in the perinuclear cytoplasm. Alkaline phosphatase begins to appear in the early months of pregnancy and gradually increases during the later months, whereas cytoplasmic basophilia, attributable to ribonucleoproteins is intensive in the first half of gestation but declines in the last half. This inverse relationship between basophilia of the syncytium and the alkaline phosphatase constitutes one of the major histo-chemical characteristics of the ageing placental villi (Wislocki and Dempsey, 1946c).

Iron-containing compounds are also found in the villi. Using the method of Hall, which demonstrates iron in loose organic compounds, Hofbauer (1905) found none such in the superficial layers of the syncytium, but an increasing number of granules were present in the deeper parts. In the mesenchyme they again decreased in number, and were altogether absent near the capillary walls. He suggests that at first the haemoglobin derivatives are in too firm combination to take on the stain, then they are further broken down and stained granules appear, and later they are again synthesised into non-stainable compounds which reach the foetal circulation. Such changes were characteristic of the first half of pregnancy. In the second half the iron-reaction of the villi was " extraordinarily slight."

Fatty substances and lipoidal droplets are always discernible in the syncytium but diminish perceptibly by the end of pregnancy (Eden, 1896 ; Hofbauer, 1905 ; Bondi, 1911 ; Ballerini, 1912 ; Wislocki and Dempsey, 1946c). Bienenfeld (1912) states that he found large quantities of lipoids in the placenta in the earlier months of pregnancy, but as pregnancy went on the lipoid content decreased. Fat droplets have also been observed in large quantities in the proliferating syncytial masses found in hydatidiform moles (Bondi, 1911 ; Ballerini, 1912 ; Wislocki and Dempsey, 1946c). Regarding the nature and genesis of the lipoid in the syncytium of the villi, *see* Chapter 16 and Wislocki and Bennett (1943).

In addition to birefringent lipoidal droplets, the syncytium of normal and hydatidiform villi exhibit a greenish fluorescence when examined with ultraviolet light and a positive reaction with Schiff's plasmal reagents, both of which are abolished by previous treatment with alcohol or acetone. According to Wislocki and his co-workers (1943, 1946c), the association of these properties in the trophoblast suggests that the syncytium and particularly its lipoidal droplets have a definite relation to the normal production of steroid hormones. Incidentally it may also be mentioned that the decrease in lipoids demonstrable histologically in the degenerating trophoblasts in pre-eclampsia and eclampsia, together with the concomitant diminution in the excretion of oestrin and pregnanediol (Smith and Smith, 1935 ; Weil, 1938 ; and others), fit in well with such an assumption. This subject is further discussed by Wislocki and Bennett (1943).

Finally, Wislocki and his co-workers (1921 and 1943) have also studied the distribution of mitochondria (which are sometimes regarded as an index of the metabolic activity of a cell) in the mature placenta of man and other animals. It

was found that while mitochondria are present in all placental tissues (foetal and maternal) they are most abundant in the syncytial epithelium forming the separating membrane between the two circulations. We have reason to suppose that mitochondria contain appreciable amounts of such substances as riboflavin, succinoxidase and cytochrome oxidase (Bensley, 1942). This has led to the postulate that respiratory enzymes may be far more abundant in the syncytium than elsewhere in the foetal part of the placenta, and that the cellular metabolism of the syncytium might be greater than elsewhere in the villi (Wislocki and Bennett, 1943). Kingsbury (1912) was the first to suggest that the mitochondria function in protoplasmic respiration and it is interesting to note that what we now know of their enzymic content, seems to lead to a similar conclusion. But, although this view has some good substantiating evidence, and serves a useful working hypothesis, it is still only a theory and must be regarded as such.

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CHAPTER 16

PHYSIOLOGY OF THE PLACENTA

By A. ST. G. HUGGETT and J. HAMMOND

I. INTRODUCTION

The study of placental physiology is at present in its infancy as compared with that of the oestrous cycle. While the structural variations in the different species have been studied by embryologists (Mossman, 1937, and Chapter 15), an investigation of the functions of the placenta presents many difficulties, not the least of which being the separation of the different parts of the placenta. For this reason much of the earlier parts of this chapter will be concerned with a form (the rabbit) in which the maternal and foetal placentae can be easily separated. In addition to being an organ for the nutrition of, and excretion from, the foetus, the placenta serves the purpose of preventing permanent attachment of the young to the mother, and moreover, during the course of evolution, has developed the production of certain hormones which play their part in the maintenance of the functions relating to pregnancy and milk production in the mother. The beginnings of the placenta are to be found in the area vasculosa of the developing egg in birds in which its functions consist mainly of the digestion, and absorption into the embryonic bloodstream, of the yolk substances stored in the egg: for an account of these functions in the egg the reader is referred to the work of Needham (1931). The early yolk sac placenta of the mammal, however, usually loses its function and atrophies early in pregnancy. In marsupials there is an attempt at the formation of a placenta from the trophoblast in some species such as *Dasyurus* (Hill, 1900), but it never comes to anything except in *Perameles* (Hill, 1897) where it does become much more like a true placenta, but is here connected with the allantois. According to Assheton (1906), the allantois is the organ of respiration and, to a certain very limited extent, of nutrition, too (i.e. water and albumen in *Sauropsida*); on the hypothesis that the mammalian ovum has been derived from a large yolked sauropsidan type, we can only suppose that it was due to the presence of the allantois that the placenta of mammals arose. Many different types of placenta exist ranging from those which consist almost entirely of foetal tissues with very little development of the maternal tissues of the uterus to those in which the maternal tissues develop to a great extent (*see* Mossman, 1937, and Chapter 15). This of necessity must affect the details of placental physiology in the different species just as the changes occurring in the uterus during the oestrous cycle also vary in the different species. The changes taking place in the uterine mucosa during the oestrous cycle are closely connected with the type of placenta which is formed by the species, for they are in the nature of a preparatory process for placental formation. Thus in the mare and sow, in

which the placenta is relatively simple, the cyclic changes occurring in the uterine mucosa are slight as compared with those occurring in the woman or the bitch in which the placenta is a more elaborate organ. The connection between the two

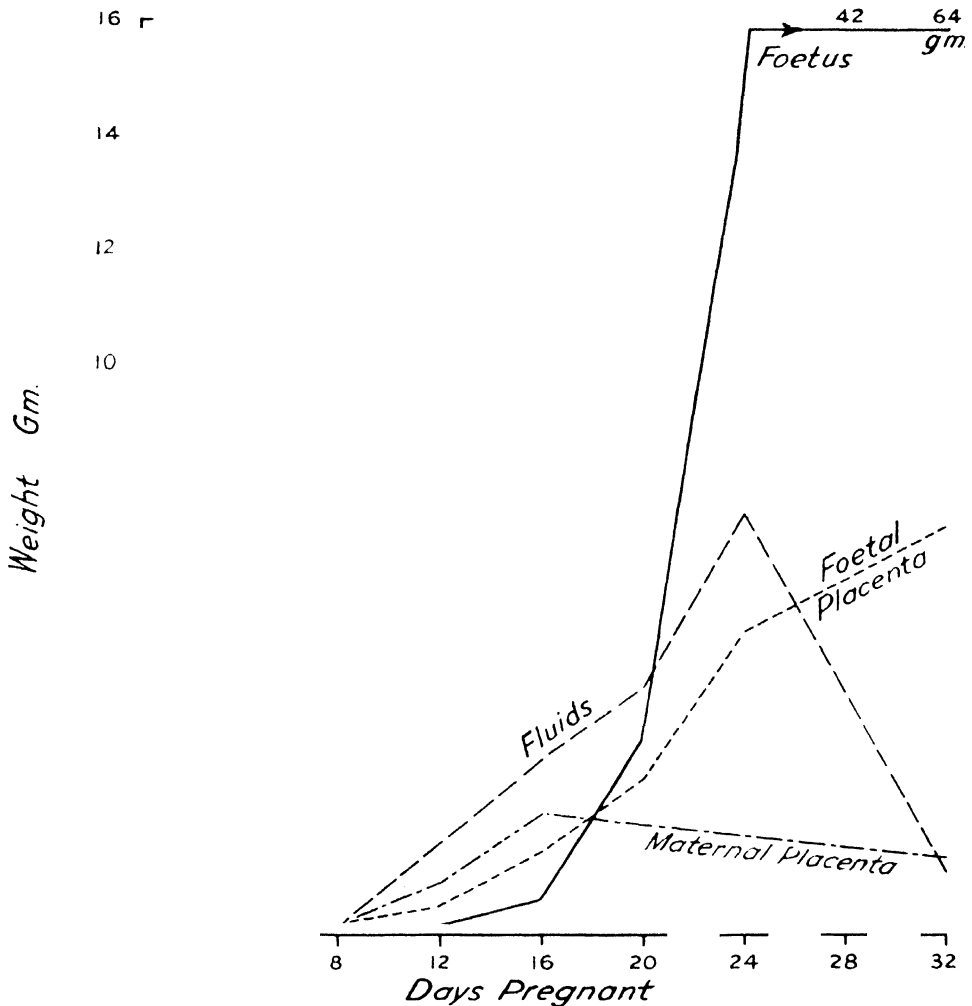


Fig. 16. 1—Growth of the uterine contents in the rabbit during pregnancy.
(From Hammond, 1937.)

is well seen in those species, such as the rabbit (Ancel and Bouin, 1910) and ferret (Hammond and Marshall, 1930), which have a pseudo-pregnancy period after a sterile coitus: the changes occurring in the uterine mucosa of these species during early pregnancy very closely resemble those which take place in pseudo-pregnancy.

II. MORPHOLOGICAL CHANGES DURING PREGNANCY

The detailed changes occurring in the placentae of different species have already been described (Chapter 15), but a general idea of the relative growths of the different tissues of the pregnant uterus during the course of gestation is also required in order to appreciate the general part the placenta has to play at different periods of gestation. In the rabbit, a species in which the maternal and foetal placentae can be easily separated from each other and from the uterus throughout

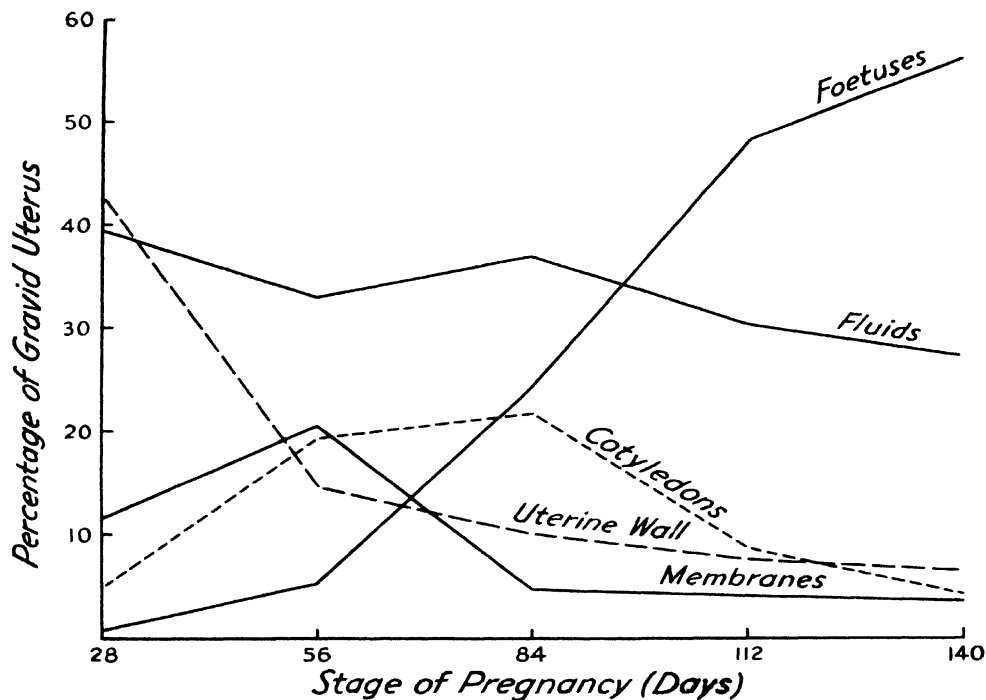


Fig. 16. 2—Growth of the uterine contents in the sheep during pregnancy. (From Wallace, 1948.)

pregnancy, the weight changes which take place are shown in Fig. 16. 1 (Hammond, 1937). Somewhat similar changes occur in the sheep (Malan and Curson, 1937; Wallace, 1948); these are shown in Figs. 16. 2 and 3. The main feature of these, and similar graphs prepared for other species, is that exceedingly little increase in weight occurs in the foetus itself during the first half of pregnancy, but that rapid growth occurs during the later stages of gestation.

The Foetal Fluids

In the early stages of pregnancy the foetal fluids increase rapidly—at a greater rate than any other part. By distending the foetal membranes they hold the embryo in place in the uterus (Assheton, 1894): as the inner tube of a bicycle tyre is held in the outer cover is how it may be described in the sheep, for in this species

the young blastocysts grow very long before distension. This distension presses the outer cells of the blastocyst—the trophoblast—firmly against the uterine mucosa and so allows the intimate contact to be made between the trophoblast

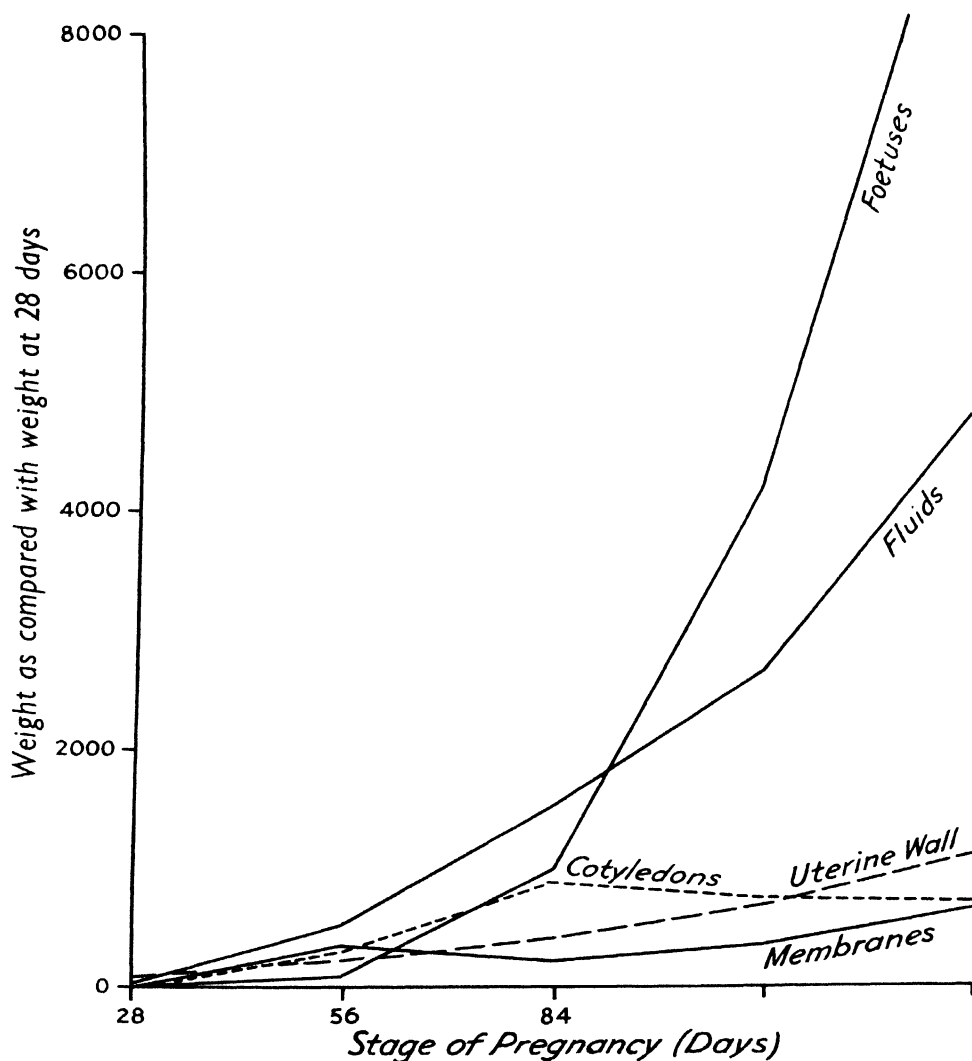


Fig. 16. 3—Changes in the proportions of the uterine contents in the sheep during pregnancy. (From Wallace, 1948.)

and the uterine epithelial cells which is necessary before the former can act on the latter and initiate the formation of the maternal placenta. In such species as the sheep (Assheton, 1906) and cow (Hammond, 1927a) where the general surface of the uterine mucosa, which is covered with the openings of glands, is studded with

projections (the cotyledons) free from glands, direct firm contact is only made on the projections and a cotyledonary placenta is formed. In the intermediate regions the secretions from the uterine glands not only serve to supply a pabulum (uterine milk) for the trophoblast cells to live on (Bonnet, 1884), but also float off the foetal membranes and prevent fixation in these regions. In the mare, which secretes large quantities of uterine milk, some of this frequently accumulates in the bottom of the uterus and gives rise to a "dead area" in the uterus where no attachment is made. In such forms as man and the guinea-pig, the fertilised ovum sinks into the mucosa, however, before distension by the foetal fluids begins. In the rabbit the rate of growth (g. per day gained) of the fluids increases up to about the 24th day of pregnancy. By so doing they dilate and expand the uterine wall. In addition to stimulating growth of the uterus through the pressure exerted (*see* p. 326), the foetal fluids form a flexible covering so that the foetus itself is protected from local external pressure either from the uterine walls or through movements of the mother. Pressure is thus equalised on all sides of the growing parts and so deformation of limb buds, etc., by unilateral pressure is avoided. When complete rat embryos with intact embryonic membranes are implanted into the chorioallantois of the developing chick the embryo develops as a whole with a correlative development and differentiation of its parts, but where embryos without embryonic membranes are implanted a total embryo is never present: instead, uncorrelated organs and tissues only are formed in the graft (Nicholas and Rudnick, 1933). After about the 24th day in the rabbit there is a sudden change, and instead of the fluids increasing further they are absorbed at a rapid rate, until in the period just before parturition merely a few drops are left (Hammond, 1935). The absorption of the fluids is not merely a passive one, but depends on the vital processes and the life of the foetus itself, for in those cases in which some of the foetuses die (*see* Chapter 21) during the 20th to 24th days of pregnancy the foetal fluids remain and wet the nest when the other normal young are born. In the case of certain abnormalities of foetal development, such as in the bulldog calf of the Dexter (Crew, 1923) and Telemark (Wriedt, 1930) breeds of cattle, the foetus fails to make proper growth although it does not die; in these cases, also, exceptionally large quantities of foetal fluids are present in the later stages of pregnancy because the abnormal embryo is failing to absorb them. It would appear, therefore, that the absorption of the fluids is due to the growth of the foetus and that one of the functions of the fluids is to form a space in which the foetus can grow by replacement of the fluids. Not in all species, however, are the foetal fluids replaced by the growth of the foetus as they are in the rabbit. The information on this point is at present very scanty. In the horse, for example, the actual weight of fluids after a preliminary rise to about the 90th day of pregnancy, falls steadily until about the 200th day, after which it continues to increase again (Hammond, 1946), the second rise possibly being due, partially at any rate, to excretion of urine by the foetus, for in the horse the foetus is born at an advanced stage of development as compared with the rabbit. In the cow (Bergmann, 1921; Hammond, 1927a) the foetal fluids increase rapidly until about the 4th-5th month of pregnancy, after which time the amount remains fairly constant, the increase, if any, occurring in the allantoic fluid, while the amniotic shows a decrease after this time, as also occurs in woman (Minot, 1892). The ewe is also similar, the amniotic fluids increase up to the 3rd

month and afterwards decrease, while the allantoic fluids increase rapidly during the last month (Malan and Curson, 1937 ; Cloete, 1939). In the guinea-pig the amniotic fluids increase rapidly up to the 44th day after which there is little or no further increase (Draper, 1920), although Ibsen (1928) records a drop at the 55-day stage with subsequent increase. What these species differences mean is not known ; they may indicate evolutionary changes with increase in the duration



Fig. 16. 4—Erosion of intercotyledonary epithelium by foetal membrane prior to formation of adventitious cotyledon in the cow : second month of pregnancy. (From Hammond, 1927a.)

of pregnancy, but before any general statement can be made a wider variety of species needs investigation.

The Maternal Placenta

The maternal placenta would appear to be a tissue formed as a result of a reaction of the uterine mucosa cells (under the influence of progesterone, the internal secretion of the corpus luteum) to the erosive action of the foetal trophoblast—in much the same way as the irritation of a wound causes the formation of scar tissue. In the cow (*see* Fig. 16. 4) the trophoblast cells can be observed breaking down the uterine epithelial cells where they come into contact with them (Hammond, 1927). Assheton (1906) has described this in detail in the sheep,

the destruction of the epithelium being brought about at first through the agency of the darkly staining binucleate cells of the trophoblast. As well as absorbing cells such as red blood corpuscles by plasmodial action it seems probable that the trophoblastic cells secrete a digestive fluid in much the same way as the spermatozoa, containing proteolytic enzymes, dissolve the "plug" in the Fallopian tube after ovulation (Yamane, 1935). If such an enzyme exists it cannot be very diffusible, however, since only in some species (sheep, Assheton, 1906) does it affect the uterine epithelium distant from the site of implantation. This erosive action of the foetal trophoblast in causing a growth reaction by the uterine mucosa can be substituted by a wound made by an incision with a scalpel or the passing of a thread through the mucosa (Loeb (1908) for the guinea-pig; Loeb (1908) and Hammond (1917) for the rabbit; Long and Evans (1922) for the rat; Parkes (1929) and Hisaw (1935) for the mouse). Mossman (1937), on the basis that the stimulus for decidual formation is not specific, but can be elicited in a number of mechanical ways, takes the view that the action of the trophoblast on the uterine mucosa is mechanical rather than through enzymes secreted by the trophoblast. From observations on the chipmunk he believes that the degeneration of the uterine epithelium does not occur until the blood supply is interfered with by some change beneath it, usually edema.

The deciduomata produced by mechanical stimulation resemble in every respect the growth produced in the formation of the maternal placenta; decidual cells are formed by enlargement of the connective tissue elements of the mucosa and small blood vessels abound (Fig. 16. 5). In order to produce such decidual growth, as also in the case of normal placental formation, it is necessary that the uterine mucosa should be sensitised by the secretion of progesterone from the active corpus luteum. The time during which this sensitisation of the mucosa exists is comparatively short. Allen (1931) has shown that in rats the sterile horn of the uterus will remain sensitised, so that deciduomata may be produced following mechanical stimulation, only up to the 7th day after ovulation. Courier and Kehl (1930), too, found that in the sterile horn of the pregnant rabbit deciduomata could be induced from the 5th to the 8th day of pregnancy, but could not be produced from the 12th to 20th days. Once produced, however, the growth of the deciduomata goes on up to about the 16th day in the rabbit in exactly the same way as does that of the normal maternal placenta (*see* Fig. 16. 1). What these time reactions are in other species has not yet been determined. Within the limits of this period of uterine sensitivity it is possible to transplant the fertilised ova of one rabbit into another. Heape (1890), for example, transferred the two-day fertilised eggs of one breed of rabbit to the tubes of another breed which had been mated with a buck of its own breed three hours before and obtained young of both breeds in the subsequent litter. Pincus (1930) similarly has reported the successful transplantation of the fertilised ova of rabbits into pseudo-pregnant rabbits at the 2nd to 3rd days after mating the does with a sterile, vasectomised buck. It is possible, too, that this capability for the attachment of the blastocyst to the uterus varies with the age of the blastocyst, for Nicholas (1933) found in the rat that when the eggs of the donor were of a later stage than those of the recipient animal only 10 per cent. developed, whereas when the eggs of the donor were of an earlier stage than those of the recipient implantation occurred in 80 per cent. of cases. Similarly he found (Nicholas and Rudnick, 1933) when implanting

complete rat embryos upon the chorioallantois of the developing chick, that the placental mechanism develops as a functional structure only in grafts taken before the middle of the 10th day. Against this view, however, is the fact that the implantation of rat or mouse ova may be delayed within the lumen of the uterus by coincident lactation when the secretions of the uterine glands are reduced and yet



Fig. 16. 5—Uterus of mouse with deciduomata.
(From Parkes, 1929.)

start development again when the uterus is reactivated. Brambell (1937), however, considers the delay in implantation is not due to an effect on the endometrium, but to some inhibitory effect on the blastocysts themselves. Several species of animals normally have a delayed implantation (Asdell, 1946); this dormant period can be reduced in the American marten by exposing pregnant females to artificial light during the winter, which suggests that activation of the pituitary gland is involved. In the rabbit, full growth of the maternal placenta is completed by the 16th day, and during this time, as Chipman (1903) has shown, its cells are being

filled with glycogen which forms a reserve until the foetal liver comes into action. The actual size which the maternal placenta attains would appear to depend on the extent of the area stimulated at the time the attachment is made, rather than on a continuance of that stimulation through an extended period of time (*see* Courrier and Kehl's (1930) results quoted above). After the 16th day of pregnancy (*see* Fig. 16. 1) the foetal placenta increases greatly in size, but this growth calls for no increase at all in the maternal placenta weight; in fact, the weight of the maternal placenta decreases after the 16th day. Barcroft (1946), too, found that the actual weight of the cotyledons (maternal and foetal) in the sheep attain their maximum weight at about the 95th day of pregnancy and then begin to decrease until near the end of pregnancy. In the sheep (Assheton, 1906) and the cow (Hammond, 1927a), the size of the cotyledons (maternal placenta) decreases from the centre of the pregnant horn uniformly to the top of the other horn, that is, from the point of greatest distension of the membranes by the foetal fluids to the tapering ends where pressure on the uterine epithelium is not so great (*see also* Reynolds's results, p. 326). In the rabbit when an embryo dies in a very early stage—before the 12th day, i.e. about the time when the mucosa loses its sensitivity—a maternal placenta smaller than normal is produced. If an embryo dies after about the 12th day the maternal placenta attains its full normal size. Such maternal placentae remain intact throughout the whole of the pregnancy just as do those of the normal young. At an early stage of pregnancy in the rabbit, attachment occurs to the two lateral, as well as to the two mesometrial folds of the uterine mucosa, but while in the latter the attachment persists and gives rise to the maternal placenta proper, in the former (lateral folds) the trophoblast after an initial attachment pulls away from it leaving a few odd cells attached in this position to the uterine mucosa (Hammond, 1935). This is probably caused by the pressure of the foetal fluids extending the thinner outer parts of the uterus to a greater extent than the thicker mesometrial parts. In the ferret (Hammond and Marshall, 1930) the same sort of thing occurs, but here where the trophoblast is detached from the decidua on the anti-mesometrial side at a rather later stage of development (three weeks) large effusions of maternal blood occur. In the rabbit the few odd trophoblastic cells which remain attached to the lateral folds of the mucosa stimulate small growths of decidual cells which persist throughout pregnancy, but never attain the size of a normal maternal placenta. The detached embryonic cells in this area, not being able to hand on the nutrients they have obtained to the foetal blood stream, grow greatly and form giant cells (Hammond, 1917). That it is the digestive activity of the foetal trophoblast which forms the basis of the flow of nutrients to the developing embryo rather than to any growth-promoting property of the maternal placenta itself is shown by the fact that if foetal tissue, such as a femur, is transplanted in the pseudo-pregnant uterus (Hammond, 1927b) absorption of the bone occurs where it comes into contact with the deciduomata or the uterine mucosa, although growth of the bone takes place where it is in contact with the uterine muscle. Nicholas (1942) has shown, too, that fertilised rat eggs transplanted into the kidney grow and differentiate much more rapidly than would have occurred in the uterus. This is probably due to the better nutritive conditions in the kidney. That the foetus can obtain nourishment from maternal tissues independently of the medium of the maternal placenta is shown by the fact that extra-uterine pregnancy may be brought about in a limited number of cases in the rat by cutting the uterine horn

after fertilisation and permitting the segmenting blastulae to fall into the abdominal cavity (Nicholas, 1931, 1934). These develop to term, but have no decidua, although they have a placental mechanism which works through vessels embedded in the mesentery. The growth of the maternal placenta appears, therefore, to be in the nature of a scar tissue formed in an attempt to repair the damage done by the foetal trophoblast. It is designed to act as a protective barrier against foetal encroachment and to prevent permanent attachment of the embryo to the tissues of the mother rather than acting as a special means of conveying nutrient to the developing embryo. That it is not necessary for nutritive purposes is seen in the fact that in some forms, such as in the mare and the pig, no appreciable maternal placenta at all exists. However, as Mossman (1937) has pointed out, in some forms such as the mole, with a well-developed decidua, the placenta does not separate, but remains in utero and is resorbed.

The relations between the maternal placenta on the one hand, and the foetal placenta and the uterus on the other hand, change during the course of pregnancy in the rabbit. Up till quite the late stages (29th-32nd day) in removing the embryos from the uterus the foetal placenta pulls away from the maternal placenta first. During the time the maternal placenta is actively growing, i.e. up to the 16th day, the maternal placenta cannot be removed from the uterus except by cutting with scissors. By the 20th-24th day, however, the maternal placenta has become loosened so that it will pull away from the uterus if the pull is directed at right angles to the longitudinal axis of the uterus; at this stage it has ceased to grow. By the 29th day the maternal placenta comes away from the uterus with traction in any direction quite easily. From the 29th day onwards an increasing proportion of placentae comes away with the maternal and foetal parts fixed together, and separation of the two parts afterwards is only effected by levering them apart. At normal birth both come away together. It is interesting to compare these changes in the maternal placenta with the effects obtained by Knaus (1926) as a result of injections of posterior pituitary extract at different stages of pregnancy in the rabbit. He found that injections up to about the 18th day had no effect on the pregnancy which continued normally; injections from the 18th to 28th day caused death of the embryos, which in the course of a day or two were aborted; and injections from about the 28th day until the end of pregnancy resulted in the immediate birth of the young. It will be seen that these critical periods on the 18th and 28th day correspond approximately to those at which changes in the maternal placenta occur; up to the 18th day the maternal placenta can only be removed from the uterus by cutting, and so contractions of the uterus do not disturb the pregnancy; from about the 18th to 28th day the maternal placenta is capable of dislodgment if pulled across the axis of the uterus, and contractions as a result of injections might well disturb the circulation and so cut off supplies of oxygen and nutrition from the embryos and cause their death. When the maternal placenta becomes closely adherent to the foetal placenta, as it does from the 29th day onwards, the injection of the extract causes immediate birth. It is probably also for the same reasons that the removal of the ovaries in the early stages of pregnancy in the rabbit causes absorption of the embryos *in situ* (as occurs also during suckling in the rabbit (Hammond and Marshall, 1925)), whereas removal in the late stages causes abortion (Weymeersch, 1911). Such absorption of the placentae occurs also when there has been a deficiency of vitamin E in the diet (Evans and Bishop, 1922;

Evans and Burr, 1927). Diakov and Křiženecký (1935) have suggested that the absence of this vitamin may act very much as removal of the ovaries does by inhibiting the formation of progesterone by the corpora lutea and so causing the atrophy of the decidual tissue. In those species of animals, such as the sheep and cow, in which the maternal placenta is not cast off at birth, it undergoes absorption after birth in much the same way, the corpus luteum in those species persisting throughout pregnancy, but atrophying at the time of parturition. If, in the rabbit, a fresh set of corpora lutea is induced at the 25th day of pregnancy (by injections of pregnancy urine extract) the pregnancy may be prolonged until the end of the life span of the induced corpora lutea (40th day); in this case, injections of posterior pituitary extract fail to induce parturition (Snyder, 1934). It would appear, therefore, that the freshly formed corpora lutea through their influence on the maternal placentae prevent detachment of the maternal placentae from the uterus. It should, however, be borne in mind that the secretions of the corpus luteum also make the uterine muscle less sensitive to the effects of posterior pituitary secretions (Knaus, 1934).

When an embryo degenerates in the rabbit the foetal and maternal placentae become more firmly attached to one another than in normal embryos, at whatever stage of pregnancy the atrophy occurs. The attachment of the maternal placentae to the uterus in such degenerate embryos, however, remains similar to that of normal embryos throughout pregnancy. The growing together of maternal and foetal placentae in the rabbit is probably due to the lack of resistance or degeneration in one or other tissue; in the case of foetal atrophy it is due to the death of the foetal placenta and the ingrowing of the maternal tissue into it, while at the end of pregnancy it is due to the degeneration of the maternal placenta and the growth of the foetal tissue into it. In contagious abortion in cattle, when bacteria break down the tissue between the maternal and foetal placentae, the same thing occurs, and this frequently leads to the retention of the foetal membranes after parturition (Pomayer, 1919).

Just before birth, changes occur in the maternal placenta. The details of these naturally vary from species to species, but in general they consist of degenerative changes. The lumen of the maternal blood vessels becomes narrowed by growth of tissue so that at parturition with the collapse of the uterus the lumen of the blood vessels is easily blocked and little bleeding occurs (Hilty, 1908; Hammond, 1927a). This is probably also assisted by the production of thromboplastic substances from the placenta (Finkelstein, 1945). When pregnancy is unduly prolonged, such as when litters of one or two are produced by rabbits which would normally be giving litters of eight or nine, the young are frequently born dead. It has been suggested that the cause of their death may be due to these degenerative changes in the maternal placenta which cut off their oxygen supply (Hammond, 1934; Barcroft, 1946).

The prenatal changes leading to birth and to changes in the maternal placenta are probably dependent on interactions in hormone production between foetal placenta and maternal internal secretions (*see* p. 327) for a maternal influence on the duration of pregnancy is shown in hybrids. The mare normally has a gestation of 335 days, but when carrying a mule foal has one of 345 days, while a donkey normally has a gestation period of 365 days, but when carrying a hinny foal has one of 355 days (Asdell, 1946).

The Myometrial Gland

During the latter part of pregnancy, from about the 22nd day onwards, special cells, named by Ancel and Bouin (1911) the myometrial gland, are to be found in the muscle layers at the base of the placenta in rabbits and some other rodents (Mossman, 1937). The latter considers them to be the result of decidual cell reaction in the connective tissue of the myometrium rather than as migrant cells from the endometrium (Hammond, 1917). They normally disappear soon after parturition. The life span of the myometrial gland is largely dependent on hormonal influences, being prolonged by progesterone and shortened by estradiol (Selye, Borduas and Masson, 1942a). Ancel and Bouin (1911) suggest that it might be a gland of internal secretion, but there is so far no evidence to support this view.

The Foetal Placenta

The foetal placenta and membranes form the organ of nutrition of the embryo and so develop early in pregnancy. While the foetus itself is yet very small the foetal placenta is quite well developed, for by its growth it prepares the way for the future nutrition of the embryo. It is suggestive that under conditions where the developing egg is transplanted into good nutritional conditions such as the kidney (Nicholas, 1942) no visible differentiation of a placenta takes place. In the rabbit, up to about the 18th day, the growth made by the foetal placenta is greater than that made by the foetus itself (*see* Fig. 16. 1), but thereafter the foetus itself increases at a far greater rate than does the foetal placenta. The maximum rate of growth (g. per day gained) occurs in the foetal placenta about the 22nd day and thereafter continues to fall gradually until the end of pregnancy, while the growth rate of the foetus increases up to the very end of pregnancy.

In the early stages of pregnancy, up to about the 22nd day, there is no definite relation between the weight of the individual foetus and the weight of its foetal placenta; but after this time the weight relationships between the two become more and more closely correlated (Hammond, 1935). Ibsen (1928) found the same thing in his investigations on the pre-natal growth of the guinea-pig, where, up to the 50th day stage, there is no correlation between the weight of the placenta and the weight of the foetus; but thereafter there is a gradual increase in the amount of correlation between the two. McKenzie and Bogart (1934) found, too, in the sheep that the number of cotyledons on the foetal placenta was closely correlated with the weight and thrift of the new-born lamb. Thus it would appear that the size to which the foetal placenta grows in the early stages of pregnancy may determine, other things being equal, the amount of nutrition which is at the disposal of the foetus for growth during the later stages of pregnancy. Barcroft (1946) found that in the later stages of pregnancy the single lamb has allotted to it about twice as much cotyledonous tissue (maternal and foetal) as a twin lamb. Up to the 70th day of pregnancy, however, he found that both twin and single lambs have approximately the same cotyledonous weight, that is, differences between the placenta weights of singles and twins begin to show up after mid-pregnancy. Whether these differences are in the maternal or foetal parts of the placenta, or both, is not yet known.

In reciprocal crosses between the large Shire horse and the small Shetland pony, in which the crossbred foal born of the Shire dam is three times as large at birth as that born of the Shetland dam (*see* Fig. 21. 13), the weight of the foetal placenta is also three times as large in the one as in the other (Walton and Hammond, 1938). By increasing the nutrition, possibly of special substances (*see* Chapter 21), available to the embryo through decreasing the number of ova fertilised in the rabbit it is possible to increase the size to which the foetal placenta will grow in early pregnancy (5.8 g. as compared with 5 g. at 24 days) and thus increase the size of the young at birth (70 g. as compared with 57 g. at 32 days). In the early stages of pregnancy in the rabbit it is the weight of the foetal placenta and not that of the foetus itself that is increased by decrease in the number of ova fertilised. Winters and Feuffel (1936) for example, found that up to about the 120th day of pregnancy there was little or no difference in weight between single and twin lambs, although at birth there are large differences. In the early stages of pregnancy in the rabbit (up to the 16th day) the foetal placenta has a slightly lower growth rate than that of the maternal placenta which it stimulates (*see* Fig. 16. 1). The growth of the foetal placenta, however, goes on in the absence of corresponding growth in the maternal placenta (after the 16th day) until by the end of pregnancy it is some five to six times larger than the maternal placenta. That is, there is no constant ratio between the two, but the ratio varies with the stage of pregnancy: for example, by weight the foetal placenta is only 36 per cent. of the maternal placenta weight at 8 days, whereas it is 71 per cent. at 16 days, 301 per cent. at 24 days, and 556 per cent. at 29-32 days. Unlike the weight of the foetal placenta, the weight of the maternal placenta bears no relation to the weight of the young born, so that one must look to the foetal rather than to the maternal placenta as being the main controlling factor in the nutrition of the embryo.

The Uterus

During pregnancy the uterus itself grows to accommodate the developing embryos (*see* Fig. 16. 6) and the growth continues throughout pregnancy. In the rabbit the extent of the growth taking place in the uterus depends very largely on the number of embryos contained in the uterine horn. The difference in the growth rate of the uterus, due to the number of contained embryos, varies greatly throughout the whole of pregnancy. Thus in the rabbit the weight of one uterine horn (stripped of all its contents) containing three embryos is 16 g. as compared with 19 g. for one containing 7 embryos at 20 days; while at 32 days a uterine horn containing two embryos weighs 25 g. as compared with 35 g. for one containing six embryos. The enlargement of the uterus during the pregnancy is thus more than a mere dilation and obliteration of the mucosal folds by the growing embryonic contents; it is one of active growth, mainly in the muscular tissue. In pseudo-pregnancy under the action of the corpus luteum very little muscular growth takes place and most of the added weight comes from growth of the mucosa. Up to the 16th day of pregnancy (corresponding to the pseudo-pregnant period) only 7 g. is added to the weight of the pregnant uterine horn, whereas in the following 16 days the increase is 25 g. in the case of a horn containing five embryos. If in the rabbit one horn is rendered sterile (by cutting the Fallopian tube; there are two independent cervixes in the

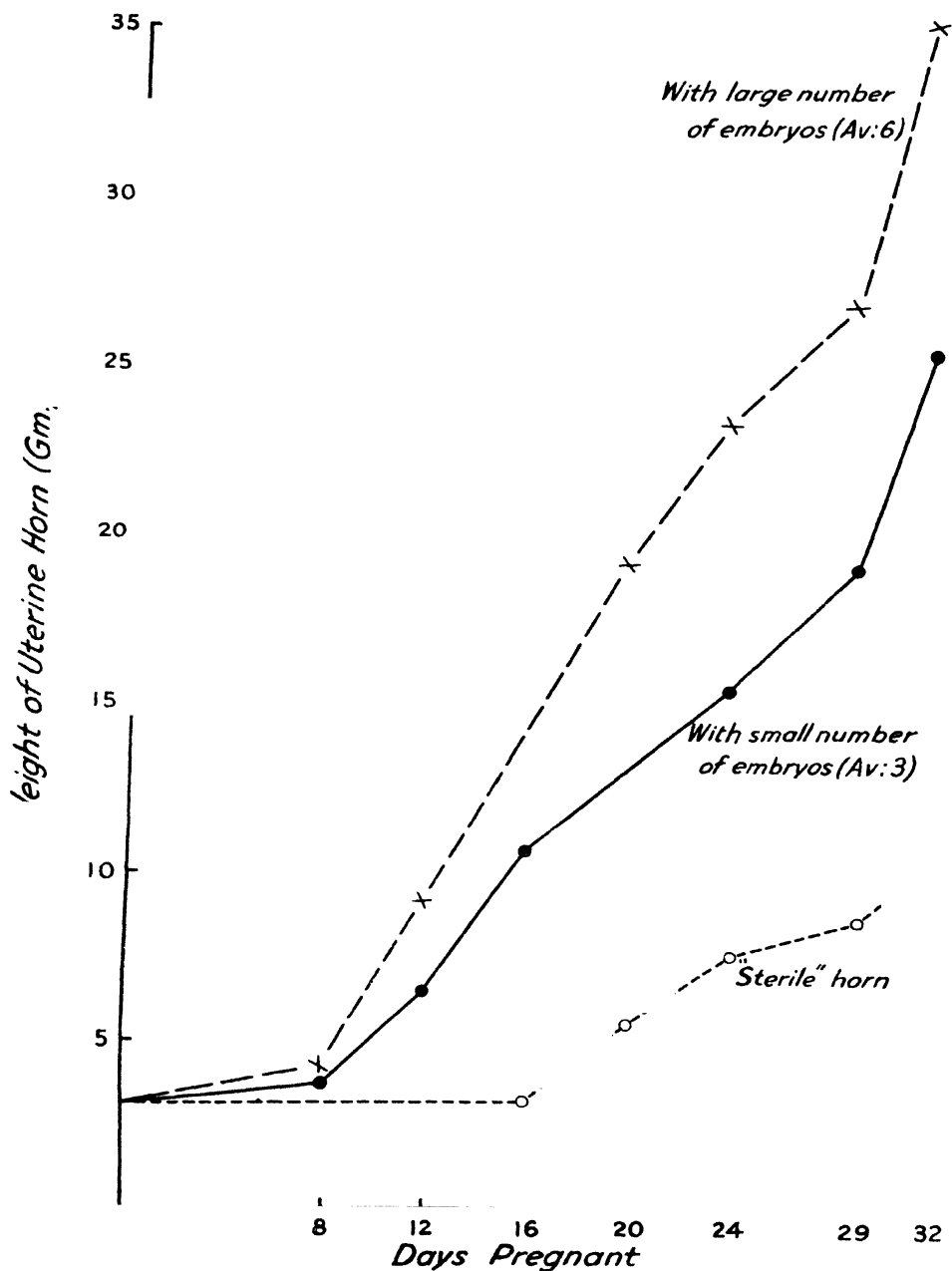


Fig. 16. 6—Growth of the uterine horn of the rabbit during pregnancy, showing the differences between horns containing large and small numbers of foetuses and "sterile" horns. (From Hammond, 1935.)

rabbit), this sterile horn grows much larger than a pseudo-pregnant horn and the muscle coat is much thicker; from weighing 3 g. at 16 days it enlarges to 10 g. at 32 days (*see* Fig. 16. 6). A similar growth of the sterile horn also occurs in ferrets (Hammond and Marshall, 1930). It is evident, therefore, that the growth of the uterus during pregnancy can be resolved into two parts. The one part, as shown by the growth of the sterile horn, is dependent on internal secretions in the blood stream; this, apparently, does not begin to take effect until about the 18th day, and up to this time the weight of the sterile horn in the pregnant rabbit is much the same as that of the uterine horn in the non-pregnant animal (Knaus, 1929). This is very noticeable when the does are killed, for up to this time (18th day) the sterile horn is long and thin, like that of the non-pregnant animal, but after the 20th day the uterus appears shortened and thickened with crinkly folds in the muscle as it contracts, like a uterine horn after parturition, this becoming more marked as pregnancy advances. This hormonal effect on the growth of the uterus has also been observed by Scammon (1926) during the development of the human embryo, in which at the 7th month of pregnancy there is an increase of growth rate in the uterus, while after birth the uterine weight declines again to the normal level. The origin of this internal secretion is not yet known, but there is some reason to believe that it is of placental origin.

The other cause of growth in the pregnant uterus is the contact effect of the embryos and foetal placentae on the uterine horn: this, in the rabbit, allowing for the hormonal growth effect at the same rate (7 g.) as in the sterile horn, amounts to 7.5 g. per embryo for horns containing two young, 4.5 g. per embryo for horns containing four young, and 4.2 g. for six young. That is, the increase in weight of the uterus with an increasing number of embryos is not constant for each one added, but obeys the law of diminishing returns. This contact effect on the growth of the uterine horn is not visible on the 8th day, but can be observed on the 12th day, that is, it comes into effect as soon as the attachment of the embryo to the uterine mucosa is made (Hammond, 1935). This contact effect of the embryo on uterine growth is a localised one in the vicinity of the attachment; moreover, its effect ceases as soon as an embryo dies and becomes degenerate. If the pregnant uterus of a rabbit is observed about the 12th day of pregnancy it will be seen that the blastocysts are by no means distributed at equal distances throughout the horns, although invariably at the latter end of pregnancy the sites of attachment are equally spaced throughout. This equal spacing is brought about by the contact stimulus to growth in the uterus; areas far distant from an implantation grow little, while those areas near the site of implantation grow rapidly. When two or three foetuses in close proximity to one another die at an early stage of pregnancy, the sites of their implantations are found crowded together and not spaced out like those of normal embryos, for on the death of the embryo the uterus in this region ceases to grow. Experimental proof of this has lately been forthcoming in the work of Reynolds and Kaminester (1936) who found that, by distending areas in the uterus of the rabbit with paraffin wax pellets, localised growth of the uterine horn was produced even in ovariectomised animals (Reynolds, 1939). Selye *et al.* (1942a, b), also found that the mechanical factor of distension of the uterus in rats caused increased muscular development of the uterus. The growth due to tension is increased by progesterone since this reduces the resistance of the muscular layers

to stretching. Coupling such experiments with injections of oestrogen, Reynolds (1937a, b) arrived at the conclusion that increase in the secretion of oestrogen as pregnancy advances gradually reduces the capabilities of the uterus to respond by growth to such contact stimuli because of the tension which the bolus imposes on the uterine wall and so leads to increased uterine pressure and eventually to birth. Thus he gives a detailed explanation of the way in which abortion is caused by injections of oestrogen during pregnancy, which explains the overriding of the secretions of the corpus luteum by oestrogen postulated by Parkes and Bellerby (1926), Courier and Raynaud (1934) and others. The latter found, too, that the amount of oestrogen required to produce abortion in the pregnant rabbit decreased as pregnancy advanced. In support of Reynolds' (1939) contention the following facts may be mentioned: in the case of mares (in which twins are uncommon and there is no corpus luteum of pregnancy after about the 140th day), the conception of twins is usually followed by premature birth, while in the case of rabbits large litters within the same strain have invariably a shorter duration of pregnancy than small litters (Hammond, 1934).

The Vagina and Cervix

The changes in the vagina during pregnancy may be referred to here, since it seems probable that, in the rabbit at any rate, the changes occurring are instigated by the secretion of oestrogen from the placenta. The enlarged vagina found after parturition is not caused merely by its stretching during the act of parturition, for during the latter part of pregnancy (after the 16th day) it has a period of active growth of tissues, particularly muscle, in preparation for the act of birth (see Fig. 16. 7). This growth not only gives the necessary muscular force for the act of birth, but also increases the size of the lumen to admit exit of the foetus. In the rabbit the diameter of the lumen (after fixation in 10 per cent. formalin) increases from 13 mm. at the 8th day of pregnancy to 47 mm. at the 31st day (Hammond, 1935). It seems probable that, since in the early stages of pregnancy (when progesterone pre-

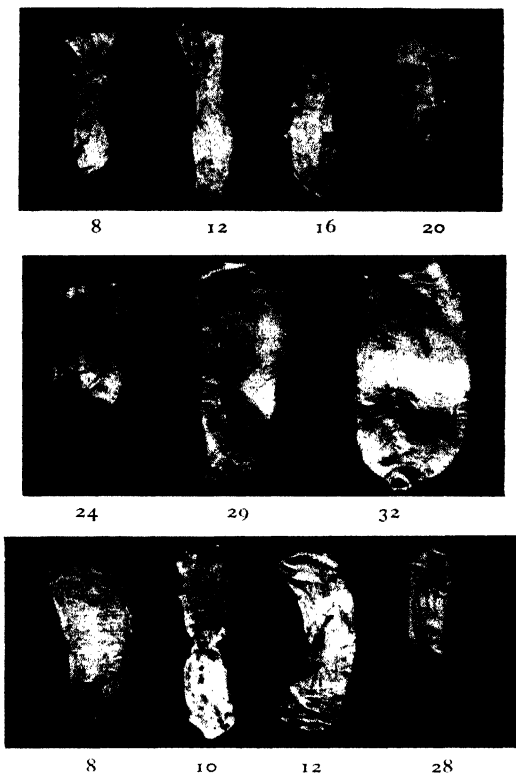


Fig. 16. 7—Changes which take place in the vagina of the rabbit from the 8th to 32nd day of pregnancy, and (bottom row) its involution from the 8th to 28th day after parturition. The numbers show the days pregnant or days post-parturition. (From Hammond, 1940.)

dominates) the size of the lumen is less than during oestrus (24 mm.), this increase in weight and lumen size of the vagina is caused by the general rise in oestrogen which takes place during the second half of pregnancy. Experimentally, similar growth changes in the vagina of the pseudo-pregnant rabbit can be brought about by the implantation of stilboestrol tablets (Hammond, jun., 1946). The growth of the cervix follows a similar course to that of the vagina and is particularly marked in those species, such as the sheep, which have a long cervix (Cloete, 1939; Wallace, 1948).

III. FOETAL NUTRITION

The Relation between Morphology and Nutrition

The supply of nutrients to the foetus is achieved in three stages: first, the period when the blastocyst absorbs foodstuffs from the surrounding fluid; next, the period of absorption by the vitelline circulation in the yolk-sac, and, lastly, the period of absorption by the allantoic circulation in the placenta. In all mammals except the Monotremata and the Marsupialia the yolk-sac system is transitory and the major route during most of intrauterine life is by the placenta and the allantoic vessels.

The Blastocyst Stage

Before the blastocyst becomes attached to the uterine mucosa it makes considerable growth, and for this purpose requires far more nutrients than are stored in the ovum. During this period nutrients are supplied by the secretions and cell debris of the uterine glands—the uterine milk (*see* Kolster, 1902, 1903a, b, c). Under those conditions in which the secretions of the uterine glands are reduced, such as in the suckling rat or mouse, the blastocyst fails to grow and remains dormant in the lumen of the uterus (Brambell, 1937). In the sheep, it is not until the 17th or 18th day that any other source of nutrition is possible, for it is not until that time that the trophoblast becomes attached to, and begins to destroy the uterine epithelium over the cotyledons (Assheton, 1906).

In the earliest stage, the blastocyst absorbs directly from cell to cell. This method is replaced later by a vascular route in the two systems, yolk-sac and allantoic. The outer absorbing layer of the blastocyst is the essential layer leading to attachment. It was called the “trophoblast” by Hubrecht (1893). The double layer formed by the fusion of the ectodermal trophoblast with the mesodermal allantois is called the “chorion.” The trophoblast has two layers, an outer plasmodium, the syncytiotrophoblast, and an inner cellular layer, the cytotrophoblast or Langhans layer. The quality of the trophoblast which enables the blastocyst to absorb foodstuffs, is also that which enables it to attach the blastocyst to the uterine epithelium. Whatever the form of attachment may be, it is always initiated by means of the trophoblastic cells, which either simply lie in close contact and tend to pierce (pig, Robinson, 1904), or do pierce the uterine epithelium (guinea-pig and man, V. Spee, 1896; rabbit, Assheton, 1895; hedgehog, Hubrecht, 1893), or bring about a general degeneration and disappearance of the epithelium (mouse, Sobotta, 1903; sheep, Assheton, 1906; cat, Duval, 1893–95).

The Yolk-sac

The yolk-sac, entodermal in structure, has the vitelline vessels running in it. In varying degrees it persists, or is aided by, or entirely replaced by the later system of the mesodermal allantois with its umbilical vessels. In the non-mammalian vertebrata (mainly reptiles) and in the Monotremata the yolk and the uterine secretions are the chief source of foodstuffs. The yolk-sac when formed, however, barely has adequate food supplies and absorbs from the uterine secretions, either directly or indirectly across the coelom. The yolk-sac is completely surrounded by mesoderm and coelom in all non-mammalian vertebrata, in the sheep and in man.

In the mammalian vertebrata the coelom is of varying size. In many the yolk-sac area vasculosa may fuse with the trophoblast to form a yolk-sac placenta. This yolk-sac placenta is always transient and of secondary importance except in the Marsupial *Perameles*, in some Insectivora and in the rodents, where it is very striking and nearly as important as the late allantoic placenta. It functions for the first ten days of pregnancy in the rat—a considerable proportion of a pregnancy only lasting 21–22 days.

The Allantois

In the mammalian vertebrata the allantois forms the later and more lasting, and in the majority of mammals, the main vascular channel. The only exceptions are the Monotremata and the Marsupialia. The fusion of the allantois with the surface trophoblast forms the chorion. The attachments of the chorion to the uterine epithelium form the true or allantoic placenta. In the primate, only the allantoic vessels and mesodermal stalk persist, the allantoic cavity never pushing out from the foetal trunk into the allantoic stalk. There is thus formed the chorionic placenta as distinguished from the allantoic placenta, in which the allantoic vessels vascularise the chorion. The term “placenta” includes both types.

Nutritive Supplies in the Pre-placental Period

What happens subsequent to attachment varies considerably in different species. In the pig the chorion is merely apposed to the uterine epithelium and forms a dome-like structure over the mouths of the glands (Assheton, 1906). (*See*, however, Chapter 15.) There is no erosion and the full six layers of the decidua and chorion persist between the two vascular streams. This is the epithelio-chorial type of placenta of Grosser's classification. In this type, therefore, the uterine glands form the main source of nutriment throughout intrauterine life, their secretions with the whole of the uterine epithelium being dependent upon the maternal blood nutrients.

In the sheep and other ruminants, the blastocyst erodes through the uterine epithelium and wallows in a lake of uterine secretions (“uterine milk” of Gamgee, 1864). In addition, the pseudopodial trophoblast cells at the base of the villi (the top of the crypt) ingest cells of the uterine epithelium, connective tissue and damaged blood vessels and blood cells (Fig. 16.8). This forms the syndesmo-chorial type of placenta (Grosser, 1927). Here, therefore, the main nutritive pabulum is the twofold supply of uterine secretion and damaged autolysing cells (epithelial,

decidual and corpuscles of the blood), together with the tissue fluids (uterine milk, plasma and exudate). In the ruminant this pabulum or "embryotrophe" (histiotrophe and haematrophe, *see* Chapter 15) is extremely important, but less so in other types. The iron is stored up in the foetal liver of the calf in the 7th to the 9th month of pregnancy (Gruszevska and Roussel, 1935, 1937). At this period the trophoblast cells at the top of the crypt of the maternal cotyledons can be seen in the cow (Hammond, 1927a) and the sheep (Assheton, 1906) engulfing the maternal red cells, breaking them down, depositing melanic pigment (as in malaria) and transferring iron to the foetal blood stream.

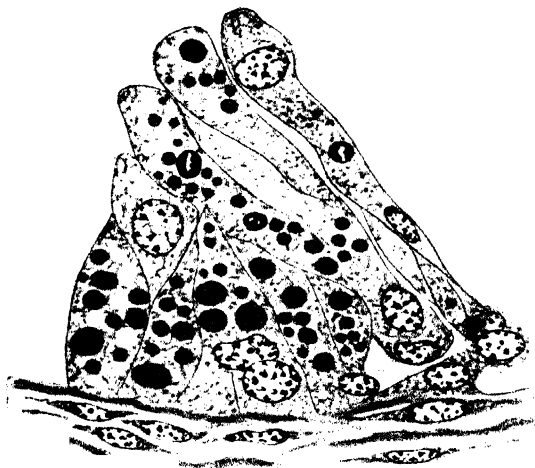


Fig. 16. 8—Columnar trophoblast cells from the base of a foetal villus of the placenta of the cow at the third month of pregnancy, to show phagocytosis. (From Jenkinson, 1906.)

As to whether this simple interpretation is entirely true must be considered in the light of the findings of Barcroft and Kennedy (1939), who have shown that in the sheep the foetus at the 80th day contains 4 g. of haemoglobin and at full term (150 days) 80 g. of haemoglobin. This big increase occurs between the 100th and 150th days. But they also show that there is a single big drop in maternal haemoglobin of 50 g. at the 40th day, that is to say two months at least before the foetus increases its own haemoglobin.

In the Carnivora the invasion proceeds further, the maternal blood vessels being eroded and considerable quantities of blood being liberated in stagnant haemorrhages (Duval, 1893). The blood so liberated autolyses and the haemoglobin forms a green pigment, uteroverdin (Lemberg and Barcroft, 1932) which is iron-free, and which is responsible for Duval's "*placenta verte des carnassiers*." This blood and the pigment therefore form part of the embryotrophe in the Carnivora.

In the primate and the rodent the erosion and autolysis go so far that the villi bathe in blood sinuses of the mother and so the haemo-chorial placenta of Grosser is formed. In the rodent this is amplified by thinning and disappearance of

patches of the trophoblast so that the two blood streams are separated by the foetal vascular endothelium, forming a haemo-endothelial placenta. The nutritional significance of this is that the placenta forms a membrane of the thinnest type across which foodstuffs can pass from the maternal to the foetal blood. In fact, at this phylogenetic stage the mother treats her embryo as one of her own organs ;

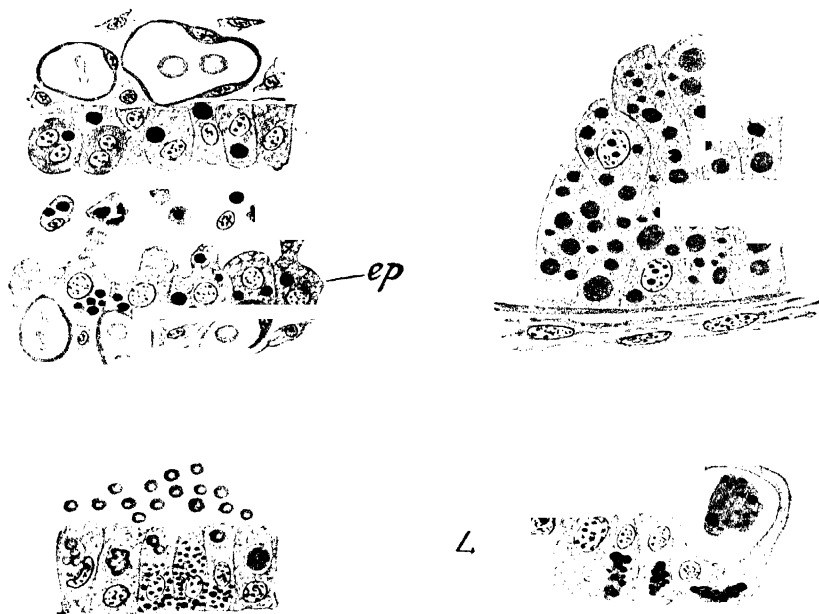


Fig. 16. g - Histology of the placenta in the cow and sheep. (From Jenkinson, 1913.)

1. Foetal and maternal tissues in a cotyledon. *tr.*, trophoblast of a villus ; the cells are absorbing fat (black). In the trophoblast are two binucleate cells. Behind the trophoblast are the connective tissue and blood-vessels of the allantois. *ep.*, uterine epithelium lining a crypt. Fat secretion is going on, the ends of the cells with fat-globules being pinched off and thrown into the lumen of the crypt. Below the epithelium are the maternal capillaries and connective tissue. 2. Columnar trophoblast cells from between the bases of cotyledonary villi. The cells are full of ingested matter (corpuscles, nuclear, and cell debris). 3. Ingestion of extravasated maternal corpuscles by the trophoblast in the sheep. The corpuscles are seen inside the cells. The cells also contain pigment. 4. Deposition of pigment after digestion of the haemoglobin of ingested corpuscles in trophoblast cells of the cow. The pigment-granules (black) are seen to be deposited on irregular masses in the cells.

on the other hand, in the Ungulata and the Carnivora, the embryo is more of a parasite dependent upon its phagocytic villi and local autolysates for food supplies. In these earlier types the materials in the maternal blood appear to be able to pass into the foetal blood, but they do not appear to form the main sources of food.

The Trophoblast

Whether the foetal nutrient is absorbed mainly from the secretions of the uterine glands or from the maternal tissues at the sites of attachment there is general agreement that the trophoblast layer of the embryonic membranes is the

tissue which is the active agent in causing this absorption. Indeed it would appear that the formation of the placenta is caused by the fact that where the trophoblast is deprived of sufficient supplies of uterine milk, such as occurs where it is pressed against the non-glandular cotyledonary area in the cow and sheep, it becomes aggressive and destroys by a digestive process not only the uterine epithelium, but also the underlying stroma cells, so eventually in many species extravasations of maternal blood are produced in which certain of the foetal villi hang, and from which consumption of maternal blood takes place directly (Figs. 16. 9 and 10). The extent to which these changes occur varies with the species. In some species, such as man, *Erinaceus* and *Cavia*, as has previously been mentioned, the whole of the nutrient of the embryo is received direct from the blood of the mother. In these types there is a complicated system of lacunae in which the

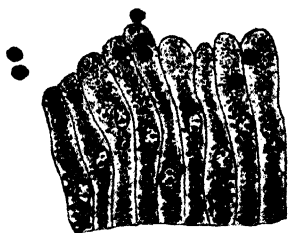


Fig. 16. 10—Ingestion and disintegration of red blood corpuscles by the trophoblast of the sheep. (From Jenkinson, 1906.)

maternal blood circulates. It is the foetal trophoblastic layer which is the active agent in not only causing digestion, but also the absorption of nutritive substances from the maternal tissues, blood stream and lymph.

The trophoblast in the primate has the power of ingesting microscopic particles of Indian ink from the maternal blood stream, but only in the early stages of gestation—it is lost by the 58th day in the Rhesus monkey (Wislocki and Bennett, 1943). Absorption of particles phagocytically as visible masses by the trophoblast is apparently restricted to the early stages of placental development not only in the primate, but also in rodents. Indian ink is not absorbed by the rabbit trophoblast and all the cases of red cells, leucocytes, uterine epithelium and decidual tissue described as being absorbed are restricted to the early stages of ovum development and to the peripheral trophoblast (Wislocki and Bennett, 1943). But it is clear that fluids with large molecules, e.g. proteins in solution, are absorbed with relative ease and it has been pointed out by Hill (1932) that much maternal tissue absorbed undergoes extensive autolysis. It appears therefore that this erosion by the trophoblast is aided by the autolysis of the substrate, the two actions being reciprocal.

Chemically the endogenous intracellular proteolytic enzyme cathepsin has been demonstrated in the placenta in both the rat and human (Goldstein and Milgrom, 1935). This autolysate of degenerative material is nominally available for trophoblastic absorption. This has been studied and demonstrated by Wislocki and Bennett (1943), who have shown that primate syncytiotrophoblast contains lipoids of endocellular origin (in their view), but at full term this syncytium is thinned out and covers the foetal capillary endothelium, while between lies a network of argyrophil fibres (precollagen) continuous with the stroma of the villi, the whole forming the “epithelial plates.” The cytotrophoblast besides showing ingested

maternal debris shows also at full term varying degrees of degenerative signs and contains glycogen, vacuoles and mitochondria, which they regard as signs of active cellular metabolism.

The Embryological Foodstuffs

It is pertinent here to review the composition of those materials which form foods, but not coming directly from the maternal blood stream or diet. They include :

1. The yolk of the egg in the Monotremata. Histologically it resembles that of the Sauropsida, but there do not appear to be any recent analyses available.
2. The prochorion.
3. The embryotrophe. This includes the secretion of the uterine glands, and also the necrotic residues derived from the decidua and maternal haemorrhages. It is of greatest importance in the Ungulata. Analysis was made by Gamgee (1864).

The principal components are :

- (a) Cellular elements—blood cells and desquamate decidua.
- (b) Fluid elements—blood, lymph and glandular secretion.

The chief chemical compounds are proteins (10 per cent.); glycogen and traces of fat (mainly intracellular); pigments which include haemoglobin and uteroverdin, a green derivative of haemoglobin peculiar to the carnivorous placenta; salts and crystals, especially calcium oxalate. The phagocytic trophoblast cells ingest these compounds so that they are visible in the chorionic villi. The evidence for the chemistry of the embryotrophe is almost entirely histological,

4. The milk of the Marsupialia and the Monotremata. Wood Jones (1939) provides an analysis of the milk of the monotreme *Echidna*. The outstanding fact is the very high percentage of solids and of the fat content.

TABLE I
COMPOSITION OF THE MILK OF *ECHIDNA*
(Wood Jones, 1939)

Specific gravity	1.023
Total solids	36.75 per cent.
Casein (?) (pH 4.7)	8.4 „ „
Albumen and globulin	2.9 „ „
Undetermined nitrogen	0.34 „ „
Lactose	2.81 „ „
Fat (Sapon. value 0.192)	19.62 „ „
Pigment	Little or none
Ash	0.78 per cent.

The Placental Barrier and Transfusion

The main function of the placenta is the transmission of materials from the mother to the foetus and vice versa. This transmission depends upon : (a) the substance transmitted ; (b) the placental structure of the animal concerned ; (c) the structure of the placenta at the stage of pregnancy in question. These

different types have been well described by Grosser (1927) and extended by Mossman (1937) in his comprehensive work on the comparative morphogenesis of the foetal membranes. These monographs provide not only an orderly account of the morphological provision of food-supplies in the pre-implantation and early placental period of intra-uterine life, but also an histological basis for permeability differences which partially correlates with the differential permeability from animal to animal, in that they classify placentae according to (a) the number of tissues between the blood streams, and (b) the diminution in layers as pregnancy advances.

Amoroso (1947) has recently described in the sow a growth of maternal capillaries within the superficial epithelium of the decidua, and of foetal capillaries within the trophoblast. This means that the two blood streams in the latter half of pregnancy are separated in the sow, not by all six layers of tissue, but by four cells only. Confirmation of this would provide a new morphological basis for the interpretation of physiological observations, somewhat at variance with that of Grosser.

The diminution in advanced placental life is shown in the syndesmo-chorial type of the goat (Ungulata). Even though the uterine glands persist throughout life, its weight increases and decreases during pregnancy; at the 10th week it weighs 354 g., at the 16th 870 g., but at the 18th it is 660 g., and at the 20th 550 g. In man, a similar weight curve is seen, but with, in addition, degenerative changes associated with the haemochorial type. Harris (1933) points out that at the 22nd week the human placenta is at the peak of fruition. After this the foetal vascular endothelium proliferates, but the placental volume, foetal blood flow and volume of amniotic fluid still increase till the 32nd week. Meanwhile, the obliterative endarteritis, the placental infarction and calcification progress, and after the 32nd week the circulating blood volume in the placenta appears to decrease. In the sheep, the blood volume in the placenta appears to be about one-third of the total volume of foetal blood in the last third of pregnancy and to keep fairly constant in this period (Barcroft and Kennedy, 1939). The decreased blood flow, the haemorrhages and the infarction are all associated with autolysis.

The Transfusion of Foodstuffs

At the same time as the placenta is undergoing these regressive changes, there are big increases in foetal weight needing big food supplies during the last portion of pregnancy. This can be regarded as a sign of one of three things, taken either singly or any two together, or all three simultaneously, namely: (i) greater demand by the growing foetus, (ii) greater food supplies by the maternal blood producing greater growth, and (iii) greater permeability of the placenta and its different layers producing greater growth.

Hammond (1944) has put forward the Theory of Partition of Nutrients to explain the distribution of foodstuffs in the maternal blood stream between the foetus and maternal tissue. It was first suggested by Child (1920) that physiological patterns such as the partition of nutrients in the blood stream between the different maternal tissues is determined by those tissues with the higher metabolic rate having priorities over those with lower metabolic rates. Applying this to the placenta, foetus and uterus, Hammond illustrates the application by Fig. 16. 11.

The foetal red cells have a greater affinity for oxygen than the maternal red blood corpuscles ; the young cells of the foetus and foetal placenta have a higher metabolic rate than the old cells of the maternal tissue. This high metabolic rate is represented in the diagram by the larger number of arrows. There is, therefore, a growth gradient between the foetus and the mother, just as there is between different tissues of the body in the animal as it grows up (*see* Chapter 23, p. 805). It follows, therefore, that with low nutrient content in the blood stream some tissues such as fat may cease growing and with still further depression of the plane of nutrition some maternal tissues (e.g. adipose tissues or muscle) may lose weight while others such as brain will still continue to grow and, in pregnancy, the foetus will grow even when the mother is losing weight. There is experimental evidence for this distribution of nutrients in adult animals (pigs, McMeekan, 1940, Pomeroy, 1941 ; sheep, Verges, 1939).

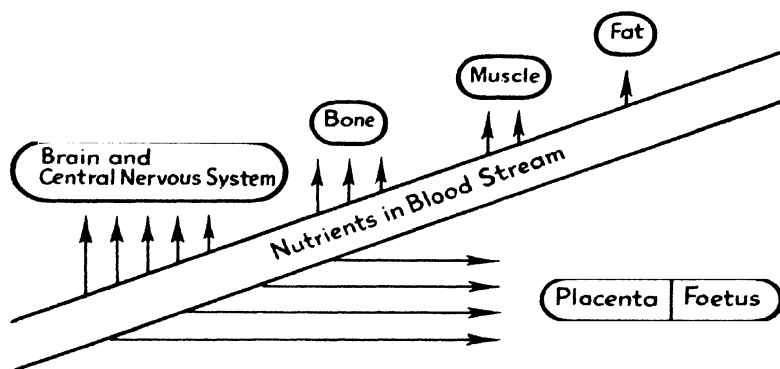


Fig. 16. 11—Priority of partition of nutrients according to metabolic rate.
(From Hammond, 1944.)

In embryos the truth of this hypothesis has been confirmed by Wallace (1944, 1945, 1948). He showed that where sheep when pregnant were fed on a low plane diet and contrasted with those on a high plane diet there was, in the foetus, a differential impairment of both tissue and organ growth ; the nervous system (with highest metabolic rate) was the least affected by lack of nutrients, whereas flesh was most affected, with bone intermediate. As with tissues so with organs ; on a low plane diet the alimentary canal was least affected and then the heart, lungs and kidneys, while the thymus, spleen and liver were most depleted by a low plane diet.

The final birth weight was dependent upon the plane of nutrition in the last part of pregnancy ; little effect resulted from the diet in early pregnancy. When twins compete with the maternal tissues they are both subnormal in weight if the plane of nutrition is low (Hammond, 1932 ; Verges, 1939 ; Wallace, 1948).

On the other hand, while extreme depletion diminishes the foetal growth when the plane of maternal nutrition is low or competition occurs, as in twins or litters (Hammond, 1932 ; Wishart and Hammond, 1933), if the plane of nutrition of the mother be pushed up to excess it does not cause extreme foetal growth beyond the normal as was shown by Eckles (1919) in the cow.

If we examine the relative concentrations of the different foodstuffs in the blood of the mother and foetus as shown in Table II, derived from Needham's *Chemical Embryology* (1931, p. 1531), we see that a substance may have a higher concentration in the maternal than in the foetal blood (e.g. glucose in man), while a different material (e.g. amino acids) may be higher in the foetal than in the maternal bloods. Occasionally, as with sodium, both maternal and foetal

TABLE II
RELATIVE CONCENTRATIONS IN MATERNAL AND FOETAL BLOODS
(after Needham, 1931)

	<i>Man</i> Haemo- chorial type.	<i>Dog</i> Endothelio- chorial type.	<i>Cow</i> Syndesmo- chorial type.	<i>Pig</i> Epithelio- chorial type.
Free amino-acid	F	—	—	—
Lipoid Phosphorus	M	—	F	—
Glucose	M	M	F	F
Na	F M X	—	—	—
K	F X	—	—	—
Ca	F	F	F	F
Inorganic Phosphorus	F	—	F	—
Cl	X	—	X	—
Fe	M	—	—	—

F = foetal higher.

M = maternal higher.

X = equal.

concentrations are the same. Perhaps the most interesting is that the glucose gradient is from the mother to the foetus in man (haemochorial) and the dog (endotheliochorial), but in the *Ungulata* the glucose travels against the gradient. It should be noted here, however, that in the light of the work of Bell (1946), Cole and Hitchcock (1946) and (Barcroft, 1946), this anomaly appears to have been dispersed. (See p. 349.) These latter are the animals with the maximum number of layers intervening between the two bloods, all active cellular layers, full-blooded and active consumers of oxygen and presumably of glucose, even at full term.

This problem of transfusion is further complicated by two things : first, other substances such as amino acids, which are freely diffusible, go if anything, against the gradient, and secondly, the placental layers store or metabolise glycogen.

Transfusion of Gases and Oxygen Metabolism of the Placenta

The passage of oxygen and carbon dioxide in and out of the blood in the tissues and the lungs is determined by diffusion under a gradient of pressure. The same conditions may apply to the placenta in considering the transfusion of gases between the two circulations—foetal and maternal. There is, however, the complication that the placenta not only is a barrier separating the two circulations and

permitting at the minimum passive diffusion, but it may also actively aid (or inhibit) gas transfusion ("secretion"). Further, it is all complicated by the fact that transfusion is only one manifestation of placental activity; it also stores materials such as fat, glycogen, iron. It has a robust structure involving protein metabolism and it is also an endocrine gland. The transfusion of gases is therefore complicated by these metabolic activities, present in the placenta, but almost entirely negligible in considerations of the pulmonary or the peripheral tissue capillary interchanges of gas.

The colour of the blood in the umbilical artery is more cyanotic than that in the umbilical vein. Following this up Zuntz (1877) alone, and later with his colleague Cohnstein (1884), showed that maternal asphyxiation caused such a lowering of the arterial oxygen content of the mother that oxygen traversed the placenta back from the foetus to the mother. This is a somewhat surprising result, since it supposes that the maternal asphyxia is so acute that, in the time during which the oxygenated blood traverses and reduces in the foetus and returns to the placenta, the maternal blood reduces to below that in the umbilical artery, and so oxygen was given up from the umbilical artery to the uterine capillaries and sinuses. The actual gas tensions were never actually measured. The experiment itself has not been repeated in quite this form, but it has been shown in the goat that maternal asphyxiation does elevate her carbon dioxide tension so that, when compared by differential tonometry, the carbon dioxide tension in the umbilical vein exceeds that in the umbilical artery; in other words, carbon dioxide passage is reversible (Huggett, 1927). This is unlikely for either oxygen or carbon dioxide if there is a secretory mechanism, but practicable on a diffusion mechanism.

The first absolute values of oxygen and carbon dioxide tensions obtained (Huggett, 1927) were incorrect, but the direct comparisons of the relative tensions showed that diffusion could occur. Similar results have been got by Haselhorst and Stromberger (1931) in man, and by Roos and Romijn in the cow (1938, 1941). In the sheep, Barcroft (1944) and his colleagues have shown that there is a difference of 50 mm. in pressure between the oxygen pressure in the maternal and foetal arterial bloods; further, that 1 mm. difference of oxygen pressure is sufficient to drive 0.1 c.c. of oxygen across the sheep's placenta in one minute.

Despite this the foetal blood only exceeds 90 per cent. saturation for a very short period in mid-pregnancy and in the last 30 days never exceeds 80 per cent. saturation and at term is only two-thirds saturated (Barcroft, Kennedy and Mason, 1940). Still more interesting is that there is a steady and progressive reduction of the maternal blood in going through the placenta; in the rabbit the uterine venous oxygen saturation has fallen to 10 per cent. at full term.

These results can be interpreted as indicating a markedly impermeable placental barrier, particularly for oxygen (Barcroft, 1944). An alternative explanation is that the simultaneous reduction in both effluent bloods—foetal and maternal—is due to active placental tissue metabolism.

There are certain points which help in increasing the gradient and the vascular area for transfusion. These briefly are the acidosis of the maternal blood in pregnancy yielding a dissociation of oxyhaemoglobin at high oxygen tension; the pronounced affinity of the foetal haemoglobin for oxygen; the morphological qualities of folded villi with superficially placed villous capillaries; the opposing flow of foetal and maternal vessels in the villi and finally the decreasing placental

layers with the approximation of the two blood streams (Barcroft and Barron, 1942). These factors are best considered in detail in Chapter 17 on "Foetal Respiration."

The important part is that the tensions permit a diffusing mechanism, but oxygen goes across with some difficulty and yet the uterine blood is remarkably reduced, suggesting active placental metabolism. The metabolism of the placenta has, however, been measured in the human (Table III) and it is of the same order

TABLE III
OXYGEN METABOLISM OF THE PLACENTA AND OTHER TISSUES

Workers.	O ₂ Usage c.c. per kg. per min.		Remarks
	Dry weight.	Wet weight or equivalent.	
Rech (1934)	—	0.71	Tissue slices
Budelmann (1939)	—	2.1	" "
Küstner and Siedentopf (1929)	12.07	6.25	" "
Wang and Hellman (1943)	88.3	45.7	at 2nd month
	63.3	32.7	" 4½ months
	53.2	27.5	" 8½ "
	28.3	14.5	" Term
Barcroft and Starling (1904)	—	40.0	Intact Pancreas
Barcroft and Straub (1910)	—	85.0	Intact Kidney

as that of other active adult organs like the kidney and the pancreas. The other interesting point is that it diminishes more or less steadily with, however, two big drops, one between the 2nd and 4th months and one in the last fortnight before term when it drops nearly 50 per cent. This drop coincides with the period of maximal thinning in the human haemochorial placenta and with the period of maximum reduction of the uterine venous blood in the sheep and rabbit. But nevertheless, beyond this one must not go, since it is clearly a source of fallacy to argue from one set of results obtained in one type of placenta to a second set applicable to a different type. It is clear that the placenta has an order of metabolism comparable with the most active organs of the adult body, but without further knowledge, it is impossible to say it is sufficient or insufficient to impair oxygen diffusion.

The application of the Warburg technique for the estimation of oxygen consumption and lactic acid production in tissues has shown that placental tissues have the high anaerobic glycolysis rate characteristic of fast-growing tissues, but only a moderate aerobic glycolysis rate, which is said to indicate non-malignancy. This suggests the trophoblast is not invading the decidua by methods analogous to those of malignant tissues (Adler, 1930; Bell, *et al.*, 1928; Warburg, Posener and Negelein, 1924).

It should be noted, however, that we have no reason to think that the normal placental metabolism is anaerobic, nor have we any evidence as to the comparative oxygen consumption of placental tissue, in different layers of the placenta, nor of the oxygen cost of different processes and functions going on within the placenta.

The Metabolism and Transfusion of Water in the Placenta

There is less reason to expect a relationship between the passage of water and its storage than there is with other substances, since it forms the basis of all cytoplasm and tissues, and of all physico-chemical processes.

In all species, the water percentage of the placenta decreases. In man, it falls by 8–10 per cent., between the third month and full term, being then approximately 85–95 per cent. (Wehefritz, 1925; Ehrenberg and Liebenow, 1924; and Watanabe, 1923). Fenger (1917) has separated the cow's placenta into maternal and foetal portions, and shown that in the former there is a constant quantity throughout pregnancy of about 82 to 83 per cent. of water, but in the latter it falls from 88 to 84 per cent. In man, it is impossible to make this separation, and the determination of these individual water contents is impossible.

When we look at the transfusion of water there are several interesting facts. First, the foetus is growing fast, and water is passing in throughout pregnancy. Heavy water (Gellhorn and Flexner, 1942) traverses the guinea-pig's placental membrane with increasing speed as gestation advances and 500 times faster than its rate of incorporation into the growing foetal tissues.

Secondly, the maternal arterial blood pressure exceeds the foetal blood pressure (both venous and arterial), though it should be noted that, so far as is known, no measurements have been made of the vascular pressures in the maternal blood sinuses of the decidual part of the placenta, nor of the capillary pressures in the foetal chorionic villi. These latter exceed the pressures of the umbilical vein. This has been determined as high as 27 mm. (Cohnstein and Zuntz, 1884) but is regarded as nearer 10 mm. (Barcroft, 1944). Yet without further missing data no conclusions can be drawn, it not really being justified to assume the maternal sinus pressure exceeds the foetal capillary pressure.

The third interesting point is the osmotic pressure of the foetal plasma. In the early measurements this neither exceeds nor is equal to the maternal plasma osmotic pressure, within the limits of experimental error (Needham, 1931, p. 1533). The mass measurements made at the beginning of this century were rough, being cryoscopic. More recent and accurate direct vapour pressure methods suggest osmotic equality between the two plasmas (sheep, McCarthy 1938 and 1946), or that the maternal osmotic pressure exceeds the foetal osmotic pressure (Barcroft, 1946). There are no known determinations in the human. The whole suggests that the explanation is not in this direction.

The fact that the osmotic pressure determinations being either equal between the two plasmas, or if anything, rather greater on the maternal side parallels the maternal protein concentration exceeding the foetal (Table IV). This being so, water should be attracted from the foetus to the mother, or, at any rate, so "bound" in the mother that it is inhibited from transfusing to the foetus.

The foetal protein osmotic pressure might even yet exceed the maternal protein osmotic pressure if certain possibilities existed (though the most accurate

TABLE IV

PROTEIN CONCENTRATIONS IN THE PLASMAS OR SERA OF MOTHER AND EMBRYO

Animal.	Foetal or Maternal.	Fluid	Total Protein. Per cent.	Albumen Globulin Ratio	Reference.
Sheep . .	M	Serum	6.24	0.5	McCarthy (1938)
	F	Serum	4.92	1.5	
Human . .	M	Plasma	7.18	1.49	Plass and Matthew (1926)
	F	Plasma	6.18	1.74	
Human . .	M	Plasma	7.16	1.0	Longworth <i>et al.</i> (1945)
	F	Plasma	6.16	1.63	

measurements have not so recorded it). First, there might be an exceptionally low molecular weight to the foetal proteins. In the horse, the serum albumen has the same molecular weight in both foetus and mother (McCarthy, 1942). There is, however, Fetuin which is such a low molecular weight globulin in the cow embryo serum (Pedersen, 1945) but it is insufficient in quantity (McCarthy, 1946). At the moment, therefore, these possibilities do not suffice either singly or collectively to explain the passage of water from the mother to the foetus.

The Transfusion of Inorganic Material

Total minerals and sodium

The total mineral content of the placenta was examined by Fenger (1917) in the cow. He estimated not only in the whole placenta, but also in the separated maternal and foetal portions and found the two inorganic metabolisms to be distinct and separate. In the early stages, there is a higher concentration of ash in the foetal part than in the maternal part. During pregnancy the maternal percentage falls but slightly, while the foetal decreases by 33 per cent. This foetal change is due mainly to the fall in the sodium chloride and the calcium, but there is a corresponding rise in the magnesium, iron and phosphorus. Though there is only a slight decrease in the total decidual mineral percentage, the magnesium and iron increase, the sodium, potassium and calcium keeping relatively constant. Throughout pregnancy the foetal sodium chloride exceeds the concentration of the maternal, though the preponderance diminishes towards full term. It would appear that sodium chloride has exceptional importance for the embryo, especially in early foetal life. In the early haemochorial human placenta, the water content, together with the sodium chloride is high. Later, in the middle third of pregnancy, the placenta increases rapidly in weight and there is with this a rise in the phosphates and sulphates of potassium, magnesium and iron. In the last third of pregnancy in the human, the predominant histological picture is degenerative absorption with decrease of calcium and iron and simultaneously the calcium, phosphorus and iron content of the embryo come (as one would expect) to exceed that of the placenta.

If the transfusion is considered by comparing the concentrations in the two bloods, there is in all recorded cases (except for some figures of Collip showing foetal preponderance of sodium chloride in the cow) almost complete equality within the limits of experimental error; the absolute value for sodium being about 350 mg. per cent. in the two bloods and the chloride values varying between 400 and 600 mg.

It would appear, therefore, that the placenta is fully permeable to sodium chloride, complete equilibration occurring between the two bloods. This free permeability to sodium chloride is, however, only relative. When it is tested by labelling the sodium chloride with the radioactive isotope of sodium Na^{24} it is found that in the Grosser-Mossman series, the permeability varies inversely as the number of layers, in the order epitheliochorial (Flexner and Pohl, 1941c), syndesmochorial (Pohl, Flexner and Gellhorn, 1941), endotheliochorial (Pohl and Flexner, 1941) and the haemochorial (Flexner and Pohl, 1941a, b and d) rabbit, guinea-pig and rat.

TABLE V

RATE OF SODIUM TRANSFER ACROSS DIFFERENT TYPES OF PLACENTA
(after Flexner and Gellhorn, 1942)

Animal.	Mg. Na transferred per gram placenta per hour at maximum.	Placental Type.
Sow	0.028	Epitheliochorial
Goat	0.43	Syndesmochorial
Cat	0.79	Endotheliochorial
Rat	10.40	Haemochorial to Haemoendothelial
Rabbit	9.25	
Guinea-pig	6.26	

These last three types at full term lose even the foetal tissues (chorion) up to the walls of the foetal capillaries (Mossman, 1937). The loss of this foetal tissue forming the haemoendothelial type is marked by a big rise in permeability to sodium chloride. Why this chorion should have obstructed sodium so intensely is not clear. Despite this increase in permeability in rodents, it takes 10 hours for 10 per cent. equilibration of sodium between the maternal blood and the foetal blood, but only 5 minutes between maternal blood and the tissues of the mother (Barcroft, 1944). The placenta is, therefore, presenting a barrier rendering transfusion slow.

One interesting result of the study of transmission of radioactive sodium was that apparently foetal growth rate is parallel to the rate of supply of sodium, except in the sow, where it outstrips the sodium supply (Flexner and Gellhorn, 1942). This finding of Flexner fits in with the view of Fenger (1917), that in early pregnancy (when foetal growth rate is maximal) the sodium chloride is particularly important to the growing embryo.

Iron

One of the more striking features of placental function is illustrated by the passage of iron. The cells of the cytotrophoblast in the cat (Wislocki and Dempsey 1946a; Duval, 1895), can be demonstrated to contain both maternal red cells (entire and disintegrating) and haemoglobin with its derivatives, the whole exhibiting the ingestion of particulate matter characteristic of the trophoblast covering the villi. In the cat and dog the only iron adsorbed appears to be by haemorrhages from the maternal vessels (Endotheliochorial placenta, Wislocki and Dempsey, 1946a). In the sow, which has an epitheliochorial placenta, Wislocki and

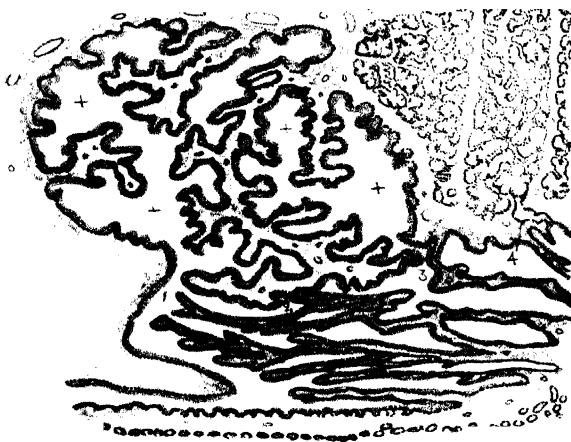


Fig. 16. 12— The labyrinth and the green border of the placenta of the dog at the fortieth day of pregnancy. To the right are two lobules of the angioplasmode which have reached the stage of complexity of the labyrinthine lamellae: to the left is the green border, the cavities of which, normally filled with blood, are indicated by a cross. (From Duval, 1893.)
1, 2 and 3, Basal lamellae of the green border; 4 basal lamella of lobule of labyrinth.

Dempsey (1946b) have found iron in the epithelium lining the uterine glands, in their secretion and in the opposed cells of the chorionic alveolae.

In the syndesmochorial placenta of the ruminants (with the exception of the cow) there are a few haemorrhages from the maternal blood vessels at the roots of the villi. The blood pigments become absorbed by leucocytes which appear beneath the uterine epithelium, some of which contain unaltered haemoglobin, others iron-containing derivatives (Assheton, 1906). The uterine "milk" includes some of these maternal red cells which become absorbed by the villi. Jenkinson (1906 and 1913) maintained that the red haemoglobin could be absorbed either in the intact red cell or alone after laking of the cell. On the whole the haemorrhages are not marked in the Ungulata compared with their distribution in Carnivora (endotheliochorial).

Jenkinson (1913) found that the haemoglobin becomes digested within the cells and that two pigments could be found, one containing iron and one free of it. The "iron-free" pigment appears as yellow-brown granules which stain the

villous cells brownish. The iron appears to have been separated off and Jenkinson surmised it was transferred to the foetal liver. The pigment has a spectrum in neutral and alkaline solutions resembling oxyhaemoglobin (two bands in the green) and in acid solution resembling acid haematoporphyrin but was not identified more exactly. The iron-containing pigment is transported to the liver.

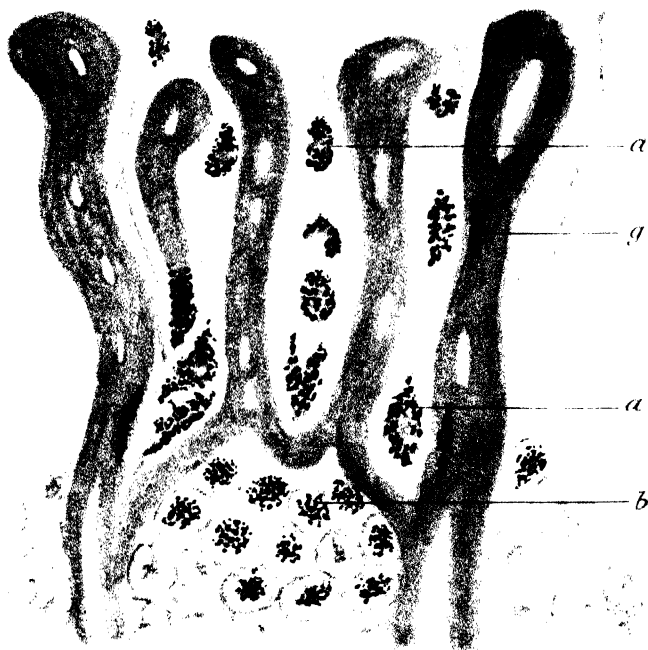


Fig. 16. 13 —Iron granules in the placenta of the rabbit at the eighteenth day of pregnancy.
(From Chipman, 1903.)

a, Iron granules in mesoblast; *b*, iron granules in multi-nucleate decidual cells; *g*, iron granules in ectodermal tubules.

The lemurs have an epitheliochorial placenta, which shows the haemorrhages of the Ungulata (Turner, 1876; Strahl, 1899) and in the trophoblast cells an unidentified green pigment, probably a haemoglobin derivative (Jenkinson, 1913), which may be identical with the green pigment in the dog (Duval, 1893) (Fig. 16. 12). This was examined by Lemberg and Barcroft in 1932 and called *Uteroverdin*. Its presence has led to the border of the placenta being called the "green border" and the placenta, the "placenta verte." It is not produced in the cat. In the leucocytes there are iron granules (siderophores) (Fig. 16. 13).

In the placenta of the rabbit the blood never stagnates, but always circulates and is always normal and never degenerates. It shows red cells in the spaces between the trophoblast cells (Maximov, 1900). Chipman (1902, 1903) has

shown that inorganic iron (stainable with dilute haematoxylin) is detectable in three different sites at different dates. It first appears at the 14th day in the foetal mesoderm as discrete granules, increasing in size and number and then disappearing slowly (though never absolutely). The second site is in the trophoblast between the 16th and 20th days and the third site is in the decidual cells close to the foetal placenta at about the 16th day, and lasts till the 24th day, when the cells degenerate and the discrete granules link up into patches.

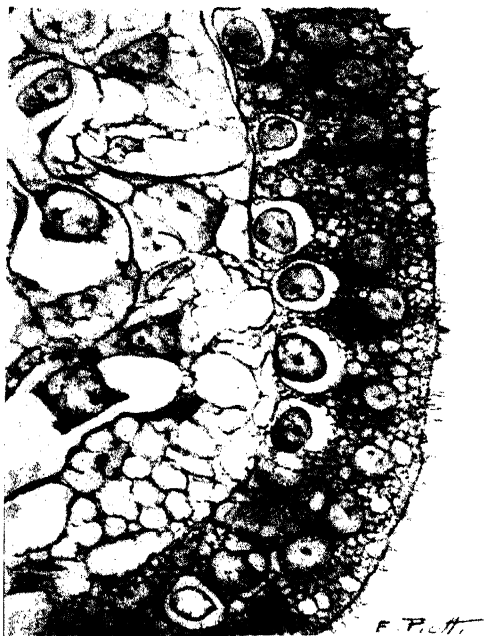


Fig. 16. 14—Chorionic villus of 3 mm. human embryo. Stained with Turnbull Blue for iron. 90 × objective, 7 × ocular. (From Dempsey & Wislocki, 1944.) The iron reaction is readily demonstrable in the intracellular matrix. Little or none in the Langhans' cells.

The physiological interpretation of this histological picture is difficult; the maternal and foetal site are in opposition; it appears in the trophoblastic before it appears in decidual cells, but without knowledge of the organic (non-stainable) iron distribution further interpretation is speculation.

In man, there is a haemochorial placenta. The early embryo has been described by Peters (1899), who found maternal red cells in the trophoblast of the early ovum, but Kworostansky (1903) and Hofbauer (1905) think haemolysis must first occur before haemoglobin is absorbed. Bonnet (quoted in Hofbauer, 1905) found eosin-staining haemoglobin in the trophoblast at points where it contacts the human blood. Hofbauer (1905) using Hall's method found loose organic iron compounds in the deeper layers of the villi, but not in the syncytio-trophoblast. They were absent around the capillary walls. He suggested that the haemoglobin derivatives are at first too firmly combined in the syncytium to stain, but more deeply they break down into iron-staining compounds, which later

(in the deep mesoderm and around capillaries and in foetal blood) become synthesised into non-staining compounds. This histological picture of Hofbauer is one which is seen in the first half of pregnancy, but in the second half, the villi show very little iron reaction.

Dempsey and Wislocki (1944) have applied a new technique to the problem of iron in the placenta in man. By micro-incineration of human placental sections, they expose all the iron in the ash, whether inorganic or free iron or combined organic iron, not stainable by ordinary staining methods. They correlated its distribution with that of the Turnbull Blue iron stain and also of the indophenol oxidase test. They show that iron occurs in two main sites, round the nuclei of the syncytium of the villi and secondly in the cytoplasm of the syncytium and also

between the cells of the cytotrophoblast, two different iron formations. The perinuclear deposits are the sites of indophenol oxidase while the cytoplasmic deposits have no respiratory enzymes. Dempsey and Wislocki therefore put forward the view that the perinuclear iron is from the cytochrome system whereas that in the syncytium and cytotrophoblast is passage iron proceeding to the foetus (Figs. 16, 14, 15, 16 and 17).

Applying this technique Wislocki and Dempsey (1946a and b) conclude that the entry of iron into the embryo is by three routes. In the pig it is absorbed through the whole chorion, but mainly by the chorionic alveolae from the glandular secretions which contain both iron and acid phosphatase. In the Carnivora it is absorbed by special areas of cytotrophoblast at the borders and base of the placenta (brown and green borders) and exclusively from maternal haemorrhages; there is no stainable iron in the uterine epithelium or lamellae. In man the iron is absorbed through the syncytiotrophoblast covering the villi and is detectable in the syncytium and stroma of the villi. The uterine glands have relatively no iron to speak of.

There is considerable direct chemical evidence of iron in the placenta. In 1921 it was shown (Wagner) that the human placental iron rose steadily in concentration till it reached 4.37 mg. at the 7th month, but fell after that to full term when its concentration was 2.55 per cent. Should pregnancy be prolonged beyond full term, then there was a further fall of iron in the placenta (Hilgenburg, 1930).

The iron appears in the liver of the foetus. It was thought (Bunge, 1893) that since there is a shortage of iron in milk, the deposit of iron in the foetal liver at full-term served as a store for the young animal while at the breast. Fullerton (1937) gives reasons for regarding this store of iron as subsidiary in the human foetal liver to the excess of iron in the blood, which he considers the major reserve of iron against infantile anaemia. The placental importance of this point is that whichever iron store is utilised after birth, it is deposited before birth by placental transfusion from the mother. Fullerton presents the evidence for showing that these foetal stores are laid down at the expense of the iron of the pregnant mother's blood and are depleted if she is on an



Fig 16. 15— Human placenta— junctional zone. 6 weeks. Stain for iron with Turnbull's Blue. 40 $\times$ objective, 7 $\times$ ocular. (From Dempsey & Wislocki, 1944.) The cytotrophoblast matrix shows an intense iron reaction; barely any in the cytoplasm of the cytotrophoblast. The pale decidual cells (lower right) show a delicately staining intracellular substance. The decidual cells show a faint blue stippling in contradistinction to the cytotrophoblast.

iron-deficient diet. Labelled iron (radioactive) when fed to pregnant women is recoverable from the human foetal blood ten minutes later (Pommerenke, Hahn, Bale and Balfour, 1942). It is widely distributed in the foetal tissues, but shows its greatest concentration in the red blood cells, and to a lesser degree in the foetal liver.

In 1931 Mackey and Goodfellow focused attention on a new aspect of the iron problem, suggesting that in human beings anaemia in the pregnant woman was associated with anaemia in infancy. They also showed that the administration of iron to the pregnant mother caused the onset of anaemia in the infant after birth, to be delayed in proportion to the pre-natal medication of the mother. Strauss (1933) proved conclusively that anaemia in pregnant women caused infantile anaemia, and that pre-natal iron supplements prevented or decreased the post-natal anaemia. In 1937, as was pointed out above, Fullerton showed that an iron-deficient diet caused depletion of the iron in the foetal liver, but in the human, unlike the dog, the main iron reserve is the foetal red cell. Clearly here the foetal iron content is influenced across the placenta by the maternal iron supplies. If the maternal diet was defective then the infant in utero was able to produce an anaemia in the mother who suffered iron loss. The infant was, however, born with an apparently full haemoglobin content but defective stores. But if the mother was given iron, the maternal blood iron depletion was not apparent—it was spared and foetal stores were supplied. In this whole business, the placenta and its iron plays no apparent part—it appears to act merely as a junction connecting two metabolic processes, in the mother and foetus, and conveying substances from one to the other. This view correlates with the later findings of Dempsey and Wislocki (1944) given above.

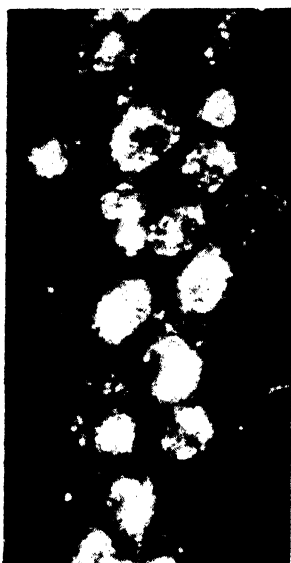


Fig. 16. 16.—Human chorionic villus 2.5 mm. Micro-incidenterated section photographed through a red (Wratten A) filter, registering red or yellow light caused by presence of iron. $\times 775$. (From Dempsey & Wislocki, 1944.) Section shows the ash distribution.

The passage of iron to the foetus being of very obvious importance it may be worth while to summarise the present view. In the sow and ruminant the chief source is the uterine secretions: in the Carnivora the chief source is the maternal blood haemoglobin iron which is ingested by the chorionic villi and the iron split off from the pigment moiety and then transferred to the foetal liver. In the haemochorial types, particularly the human, the major source of iron is the maternal blood but not merely the haemoglobin but also iron food-stuffs in the blood stream. These latter apparently can transfuse very quickly. The placental iron appears to be mainly of two types, the passage iron *en route* from mother to foetus (which barely, if at all, enters into the placental metabolism) and the iron of the placental metabolism, mainly in the cytochrome oxidation system.



Fig. 16, 17—Human placenta, chorionic villus and cytotrophoblastic column (same placenta as Fig. 16, 14). Microincinerated and photographed through a red (Wratten A) filter. $\times 280$. (From Dempsey & Wislocki, 1944.) Shows dense deposits of ash in the matrix between the cytotrophoblast cells and also in the syncytium of the chorionic villus.

Copper

In the first part of pregnancy there is a rising content of copper in the foetal liver in humans and pigs. (Wilkerson, 1934 ; Ramage, Sheldon and Sheldon, 1933.) In the pig its concentration keeps fairly constant in the latter months, but in the human it rises steadily towards full-term, and, in fact, achieves an hepatic concentration at term double that in the adult liver. This is regarded by McFarlane and Milne (1934), who studied its deposition in the chick's liver, as evidence of free passage of copper across the haemochorial placenta and as equal evidence of absence of this free passage across the epitheliochorial placenta of the pig.

Manganese

This, like iron and copper, appears in the human foetal liver (Ramage, Sheldon and Sheldon, 1933), and in the calf (Gruzewska and Roussel, 1937). Its mode of passage is unknown—it does not, however, accumulate like iron.

Calcium and phosphorus

The passage of calcium is marked by certain points : it is present in the placenta and increases as the placenta enlarges towards term ; there is calcium oxalate in certain masses of tissue floating in the amniotic fluid. These masses called hippomanes have calcified glycogen deposits in them and are of unknown significance (Jenkinson, 1913). Further, the calcium (and the inorganic phosphorus) is one

of the substances required by the foetus, yet, however, existing in the foetal blood in higher concentration than in the maternal blood, showing that their passage is against the gradient. Lastly, there is a definite demand by the foetus for these substances to such an extent that if they are deficient in the maternal food supply in pregnancy they appear to be depleted from the maternal tissues (Sherman and Macleod, 1925 ; Garry and Stiven, 1936).

Wislocki and his colleagues, Bennett and Dempsey, in a series of papers in 1944-5-6, using histochemical staining of the placenta in man, rodents, carnivora and the pig, suggested that calcium was detectable (Bodian's argyrophil stain and micro-incineration) and also phosphatase, both acid and alkaline types. To the phosphatases they attribute several functions : first, an inverse relationship with ribonucleoproteins which will be considered in conjunction with the protein metabolism of the placenta ; second, the usually attributed calcifying function of phosphatase into which they suggest the glycogen of the placenta enters. The hypothesis is put forward that glycogen liberates hexosephosphate which forms a substrate for the phosphatase—an analogy with the calcification of bone. In the ageing placenta (human and rodent) calcium is mainly deposited at the junctional zone when ageing. In the cat, traces of the argyrophil reaction are detectable in the placental giant cells (regarded by them as decidual). In these there is temporary storage of calcium in apparently physiological amounts in the stroma of the chorio-allantois throughout most of gestation. Irrespective of whether such calcium deposits are physiological or degenerative, they are usually associated with demonstrable glycogen and phosphatase—two substances which appear to be essential for calcification (Wislocki and Dempsey, 1946b).

There is a direct relation between the calcium utilisation in pregnancy and the calcium content of the diet. If it was decreased in the diet of a pregnant bitch, there was no deficiency in the embryo (Dibbelt, 1910 ; Zuntz, 1919), but in the pregnant rat there is a disappearance of calcium and phosphorus from the tissues of the mother (Sherman and Macleod, 1925) and extra calcium is required to prevent this, both in rats and humans (Tøverud and Tøverud, 1929a and b). The ossification of the human foetus is dependent on the dietary content of calcium and phosphorus ; if it is low, rarefaction of the bones and teeth occur (Booher and Hanseemann, 1931). To obtain adequate protection against this rarefaction the human calcium intake needs to be increased from 0.66 g. per day to 1-2 g. and phosphorus from 1.23 to 2 g. daily (Garry and Stiven, 1936). While Dibbelt (1910) and Zuntz (1919) showed foetal calcification proceeds normally on a low maternal intake of calcium, this apparently is only true if adequate vitamin D is present—if this is deficient then calcification is defective (Sontag, Munson and Hoff, 1936). It is clear therefore that the foetal calcifying mechanism can influence the maternal endogenous metabolism of the bones across the placenta. This can be looked upon as exemplifying Hammond's Theory of Partition of Nutrients. The pool of calcium and phosphorus existent between the high metabolic rate growing bone, the foetal blood, the placenta, the maternal food and the low metabolic rate maternal bones is utilised so that calcium and phosphorus are drawn continually to the foetal skeleton. This occurs despite the fact that practically all measurements demonstrate that the concentration of inorganic calcium and of the inorganic phosphorus and all organic phosphorus and the total phosphorus in all species (with the exception of a few measurements in

man of lipid phosphorus) is higher in the foetal than in the maternal blood or plasma (Needham, 1931, pp. 1521 and 1528). In the last part of pregnancy the rate of deposition is immensely accelerated. At this stage the placental membrane is thinner; nevertheless, the adverse gradient is maintained and the calcium passage from mother to foetus continues and the maternal bones become rarefied if food supplies are deficient. As has been said, phosphorus, whether organic or inorganic, exists in higher concentration in the foetal than in the maternal blood in all species including man, though a few measurements of the lipid phosphorus in man show a higher concentration in the maternal blood (Needham, 1931, p. 1521). Phospholipids appear to pass very slowly, their passage when labelled with radioactive phosphorus having been observed by Nielson (1942). He advanced the hypothesis that it traverses the placenta as a small molecule and that the foetus later synthesises it to phospholipid.

The Transfusion of Carbohydrates

In all mammals except the Ungulata the blood sugar concentration is higher in the maternal than in the foetal blood. The main findings in the literature are shown in Table VI, derived from Needham (1931), p. 1525. This ungulate condition may be accounted for by saying that in the six-layered epitheliochorial or the five-layered syndesmochorial placenta sugar has been thrust across by active secretion, but that it need not be in the thinner endotheliochorial and haemochorial placentae. This is, however, hypothetical and there is no absolute evidence of an active secretory mechanism in these placentae.

In the human, the maternal blood sugar is always greater than the foetal in the normal individual. The foetal blood sugar is constant at any one age, but steadily increases towards full term (Snyder and Hoskins, 1928b). In twin foetuses the two sugars are approximately equal, but both are less than the maternal sugar (Slemons, 1919; Lawrence, 1929). Where the maternal blood sugar is elevated as in anaesthesia, diabetes or diabetic coma, the foetal sugar is elevated likewise, but is always less than the maternal by considerable amounts. Ketteringham and Austin (1939) showed that in the second stage of labour (during expulsion of the child) intravenous injection of glucose elevated both the maternal and foetal blood sugars to values averaging 376 mg. and 241 mg. respectively.

There is one exception to the usual ungulate findings of foetal blood sugar values exceeding those of the mother in that Cohnstein and Zuntz (1888), when producing experimental hyperglycaemia in the pregnant sheep, always found the foetal blood sugar was below that of the mother.

While, therefore, the balance of the evidence suggests that sugar passes from the mother to the foetus by a diffusion mechanism, the case is not clear-cut for all mammals. This doubt that it is a simple physical process is due to a number of points.

First the thickness of the placental membrane varies in different mammals and especially in the same species at different placental ages. Second, Claude Bernard in 1859 demonstrated by iodine staining the presence of deposits of glycogen in the rabbit's placenta. Third, Paton, Watson and Kerr (1907) thought that there was glucose in the maternal blood, but fructose in the foetal blood and amniotic fluids. For these reasons alone one cannot dismiss carbohydrate transfusion

TABLE VI

RELATIVE CONCENTRATION OF BLOOD SUGAR IN MATERNAL AND FOETAL BLOODS
(after Needham, 1931, p. 1525)

Placental type.	Animal.	Maternal. Mg. Per cent.	Foetal. Mg. Per cent.	Remarks.
Epitheliochorial	Pig	100·0	139·0	Aron (1924)
Syndesmochorial	Cow	100·0	110·0	Aron (1924)
Endotheliochorial	Dog	110·0	65·0	Aron (1924)
"	"	139·0	123·0	Morris (1917)
Haemochorial	Rabbit	125·0	36·0	Aron (1924)
"	"	140·0	103·0	Brandstrup (1929)
"	Rat	112·0	69·0	Corey (1928, 1930)
"	Guinea-pig	107·0	60·0	Aron (1924)
"	"	113·0	68·0	Kito (1919)
"	Man	132·0	110·0	No anaesthetic, Morris (1917)
"	"	180·0	125·0	Anaesthesia, Morris (1917)
"	"	112·6	84·4	Bell, <i>et al.</i> (1928), Maternal artery and umbilical vein
"	"	106·0	75·2	Maternal vein and umbilical artery, Bell, <i>et al.</i> (1928)
"	"	120·0	80·0	Lawrence (1929)
"	"	180·0	125·0	Umbilical vein, and Lawrence (1940)
"	"	"	75·0	Umbilical artery, Lawrence (1940)
"	"	800·0	220·0	Diabetic Coma, Offergeld (1908)

lightly as one of mere physical diffusion until one considers the significance of these phenomena.

The problem of fructose is curious. Bernard (1859) stated as a result of polarimetric measurement, that the rabbit's foetal blood sugar was fructose. In 1907, Paton and his colleagues confirmed this, using Seliwanoff's reagent, and extended it to the allantoic and amniotic fluids in the rabbit and the sheep. Since then, glucose has sometimes been found, though in most cases fructose has been confirmed using either the polarimeter, the Seliwanoff test, or the methylsazone formation (which distinguishes the two sugars).

Since then, fructose has been demonstrated in the amniotic fluid of the whale (Takata, 1922) and of the chick (Yamada, 1933); in the foetal blood of humans

and of goats (Orr, 1923). Still more, van Creveld and Ladenius (1928) showed fructosuria in pregnancy and Okamura (1933, 1939) showed traces were present in the normal blood of a woman, but there was an occasional elevation in pregnancy.

The problem has been clarified by Barcroft's colleagues, Bacon and Bell (1946) and Cole and Hitchcock (1946). Bacon and Bell have not only prepared both methyl-phenyl-osazone and the diacetyl derivative of fructose, neither of which can be got from glucose, but also have isolated pure fructose from the sheep foetus (Barcroft, 1946). Cole and Hitchcock (1946) have estimated the total reducing sugar in the sheep's amniotic fluid and the foetal and maternal bloods (venous and arterial) and simultaneously the fructose (by Roc's method, 1934) in the same fluids; the difference between the total sugar and the fructose being assumed as glucose. The results are shown in Table VII.

TABLE VII
MG. HEXOSE PER 100 C.C. FLUID
(After Cole and Hitchcock, 1946)

Foetal age (days).	72		102		121		122		144	
	G.	F.	G.	F.	G.	F.	G.	F.	G.	F.
Uterine artery	79	2	73	1	91	1	68	1	60	
Uterine vein	55	3	58	1	74	2	83	1	41	1
Umbilical artery	38	143	25	106	64	134	19	50	16	54
Umbilical vein	—	—	37	108	12	146	37	47	24	57
Amniotic fluid	—	—	113	124	57	363	136	90	200	103

G. = Glucose. F. = Fructose.

In part, these quantitative results confirm Paton, Watson and Kerr (1907), but the case is tightened up by the technique observed. The outstanding points of note are the presence of both glucose and fructose in the foetal blood with practical absence of fructose from the maternal blood; the excess of glucose in maternal blood over that in the foetal blood, and the fact that approximately 70-80 per cent. of the foetal blood sugar is fructose. From this it is clear that while glucose can pass across the placental barrier, fructose cannot. There is also no clear-cut correlation of any of these figures with foetal age.

In connection with fructose, there are two points of interest. Mann (1946) has shown that the sugar in seminal plasma is fructose. Dickens and Greville (1932a, b, c), have shown that all tissues (normal adult, embryonic and tumour) can utilise both glucose and fructose aerobically, but anaerobically all can break down added glucose (glycolysis), but only some embryonic and some tumour tissues are able to convert added fructose to lactic acid (fructolysis). Which embryonic tissues and membranes effect this fructolysis appears to be haphazard both in degree and actual activity. There was no correlation with growth rates or embryonic layers.

Beyond saying that fructose is a peculiarly foetal product one cannot venture more as to its function at this stage.

These results need to be correlated with the presence of hyaluronic acid in Wharton's Jelly (Meyer and Palmer, 1936; McClean and Hale, 1941). This is a polysaccharide containing N-acetyl glucosamine and glycuronic acid, and is present as a major component in Wharton's Jelly. Its presence in the sheep's cord was confirmed by Barcroft and McAnally (1946). It can, therefore, be expected in the foetal part of the placenta. It decreases the barrier offered by cellular and other membranes to diffusion of materials across the membrane and in this connection, it has been demonstrated that certain colloidal dyestuffs pass freely



Fig. 16. 18--Glycogenic areas of the rabbit's placenta at the twelfth day of pregnancy. (From Chipman, 1902.) *fp*, Foetal placenta, containing no glycogen; *ir*, intermediary region; *rs*, region of uterine sinuses; *ss*, uterine sinuses with perivascular sheaths of uninucleate cells rich in glycogen; *g*, glycogen granules in multinucleate cells; *m*, muscular wall immediately above which, at a later date, the zone of separation, containing glycogenic decidual cells, is differentiated.

along the cord in Wharton's Jelly towards and into the foetus. This may form a pathway from placenta to foetus, other than via the blood vessels (Barcroft, Danielli, Harper and Mitchell, 1944).

Considerably more is known about the glycogen deposits of the placenta. It has been investigated by two methods, histological staining by iodine or Best's Carmine method and by chemical estimation by Pflüger's method.

After Bernard's demonstration of glycogen in the rabbit's placenta, it was shown to be in two areas, below the villi and in the deeper tissues of the placenta (Godet, 1877) and to appear in the decidua at the 8th day (Maximov, 1900). Histologically, Chipman (1902, 1903) thought its maximum deposition in the rabbit was at the 16th day (Fig. 16. 18); this date, however, was not confirmed by the chemical method used by Lochhead and Cramer (1908), who showed it developed

its highest concentration (5.5 per cent.) at the 21st day and then diminished towards full term. Both Chipman and the later pair of workers thought it did not appear in foetal tissues, only in the decidua and the maternal tissues round the villi (the "distal" and "proximal" glycogens respectively). Jenkinson (1913) doubted this restriction and Szendi (1934) confirmed this doubt showing it was in the foetal placenta. Jenkinson (1906) had previously shown it to be present (histologically) in the ungulate (cow and sheep—syndesmochorial) placenta in the trophoblast, mesenchyme and the amniotic stratified epithelium, as well as in the uterine epithelium and the uterine "milk." This foetal deposition he showed also to be present in the

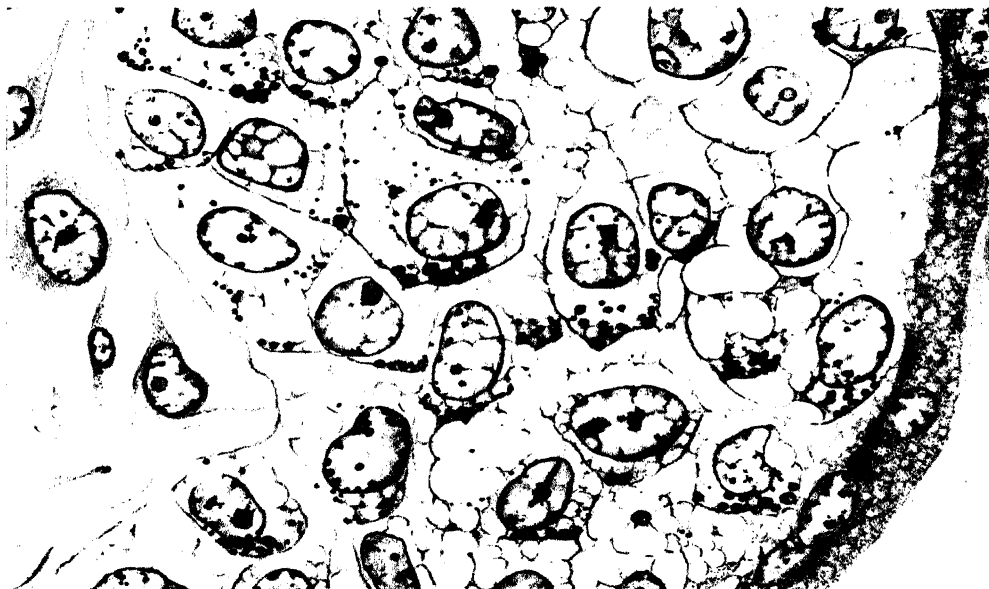


Fig. 16. 19.—Placenta of a Rhesus monkey, 58 days. Stained for glycogen by Best's carmine. $\times 10$. The cytotrophoblast, especially when it is vacuolated, contains large quantities of glycogen. The less vacuolated varieties of the cytotrophoblast, including Langhans' cells, contain less glycogen. Only traces of glycogen, at best, are ever seen in the syncytium. The cells of the decidua also contain large amounts of glycogen. (From Wislocki and Bennett, 1943.)

mouse (1902); Driessen (1907), however, showed that in man it was decidual and trophoblastic. In the third type of rodent, the rat, it is in the trophoblast as well as decidua (Goldmann, 1912) and here it is found mainly in the reticularis of the foetal placenta which is almost entirely ectodermal, but only traces are detectable in the ectodermal cells of the labyrinth. This distribution is not altered nor its histological concentration increased or diminished by foetal death with continued growth of the placenta (Huggett and Pritchard, 1945a, b). In man, it appears in the decidua and in the cell columns joining the decidua to the trophoblast (chorio-basalis), but more appears in the syncytium and cytotrophoblast (Fig. 16. 19).

Wislocki and his colleagues have put forward original views on the rôle and deposition of glycogen in the placenta. Dempsey and Wislocki (1945) compared

its distribution in the human placenta with the distribution of the indophenol oxidase and with the tissue's oxidation—reduction potential determined histologically. They decided a high respiratory metabolism was associated with a poor glycogen deposition and vice versa, whether due to defective blood supply or to paucity of oxidising enzymes. In 1946, they compared its distribution with that of alkaline phosphatase and found there was a close correlation in nearly all species—human, rodents, cat, sow—which they investigated. Adopting the Cori cycle hypothesis, that a phosphorylated intermediary is a necessary antecedent to glycogenesis, they hypothesised that the formation of glycogen is preceded by dephosphorylation of the hexose phosphate which condenses to form glycogen, which requires a phosphate splitting enzyme such as alkaline phosphatase. Phosphatase should therefore be associated with glycogen. In man, they found alkaline phosphatase in the endometrial capillaries and glycogen in the epithelium; in the cat, glycogen and alkaline phosphatase occur together in the deep uterine glands; in the sow, glycogen is found in the chorion cells, but is absent in the endometrium, while alkaline phosphatase is in two localities, the endometrium and the apical borders of the chorion cells.

When estimated chemically, there is some lack of parallelism between the results obtained and the histological staining. This is a common finding in estimations of glycogen in other tissues. As has been said above, Lochhead and Cramer found the maximum quantity appeared at the 21st day of pregnancy in the rabbit, and then it diminished. They found the concentration appeared fairly constant and it was fairly stable. Pronounced diminution was found only if they gave phlorhizin regularly for ten or twelve days. This caused diminished foetal growth with which was associated a diminished glycogen concentration. They also extracted an amylase from the placenta. This, interestingly, was in greatest concentration in the foetal portion and not in the decidua, where the glycogen is demonstrable in greatest concentration. Macda (1923) also extracted an amylase from the placenta (human).

The properties of placental and hepatic (maternal) glycogen in the rabbit differ materially (Huggett, 1929). Though it is almost entirely restricted in this species to maternal tissues it is more stable than maternal hepatic glycogen. It is independent of variations in the blood sugar caused by sugar infusion, by adrenalin and by biological doses of insulin which alter the hepatic glycogen. It is, however, diminished by factors diminishing body weight by increasing katabolism of the tissue, such as phlorhizin, thyroid administration or gross repeated doses of insulin. Davey and Huggett (1934) found that aerobic autolysis of rabbit's placental breis led to the formation of glucose and polysaccharides, but not of lactic acid. These results amplified and confirmed Ishikawa (1928), but not Harada (1916) who, using human placenta, said lactic acid was formed. Corey (1935) working on the rat confirmed the stability of placental glycogen and its independence of endocrines.

Szendi (1934) compared the chemical and histological methods of study in rats, rabbits and man. He found the rabbit's "proximal" glycogen of Lochhead and Cramer was a deposit in the trophoblastic portion known as the "ectoplacental cone," that it was, however, only about a tenth of that in the decidua but its peak of concentration was earlier, about the 17th day, and it was waning in concentration when the decidua glycogen was increasing still. Its maximal concentration, too,

was much less, 1 per cent. as against 5.5 per cent. in the decidua, or taking the placenta as a whole, foetal and maternal, 3.5 per cent. at the 21st day.

In man, the placenta achieved its highest concentration much earlier, not as in the rabbit and rat at about two-thirds through pregnancy, but at the 20th day from conception when there is 2 per cent. in the foetal placenta and 4 per cent. in the decidua (Szendi, 1936). By the 1st-2nd month it has diminished, averaging 0.37-0.5 per cent. in the placenta (Szendi, 1934; Eufinger, 1932), with subsidiary peaks at the 5th and 7th months.

Szendi concluded that in the placenta the deposition of glycogen varies at different ages and in different sites in the same species, quite apart from species differences. He thought that the quantity of glycogen in the cells of any one spot at any one time, is a measure of the activity of those cells, great metabolic activity being associated with quantities of stainable assayable glycogen. This view is in direct opposition to that put forward by Dempsey and Wislocki (1944) who considered the deposition of glycogen to be related inversely to the metabolic activity of the cells in which it appears.

Two things are clear about the placental glycogen. The first is its curious properties—its variable concentration at different ages, its dissociation from glycogenase, its association with phosphatase, its difference in foetal and maternal placental tissues, its extreme stability and relative independence of blood sugar or hormonal influences, its apparent direct relation to metabolic rate (Szendi) and to the inverse of the metabolic rate (Dempsey and Wislocki), or rather oxygen metabolism. The second thing is that it has no relation to the transfusion of sugar and the levels of sugar in the mother and foetus, which fluctuate rapidly in parallel and in no way affect the glycogen. This glycogen appears to be an endogenous metabolic product associated with the structure and growth of the placenta as a transfusion agent. The clue to this may well lie in the views of Wislocki and his colleagues. But considerable further work remains to be done upon its properties both chemical and biological. Claude Bernard's idea that glycogen in the placenta may be deputising for liver glycogen is attractive, supported by evidence (mainly its disappearance as foetal liver glycogen increases), but cannot as yet be regarded as proven. It is somewhat negated by the relative stability of placental glycogen and the independence of the blood sugar variations.

The Problem of "Malignancy" in the Placenta

Warburg (1923) put forward the view that malignant tissues differ from normal tissues in that they can form lactic acid from glucose aerobically (glycolysis). Using tissue slice technique he studied the oxygen consumption, and lactic acid production in the presence of glucose. He denoted the corrected aerobic glycolysis by the symbol U , which he found to be negative with normal tissues, mildly positive with benign tumours and markedly positive with malignant tumours. Applying the Warburg technique to placentae, Murphy and Hawkins (1925) found rat placenta to have $U = +0.3$ ("benign"); Loeser (1926) found young human placenta to be -4.2 and full-term human placenta $+10$, suggesting a drift towards a metabolism similar to that of a malignant type. Bell, Cunningham, Jowett, Millett and Brooks (1928) followed this up and found human placenta without chorionic villi were definitely negative (-2.5), but with chorionic villi

present were positive (+ 0.7 to 1.0). Since the villi formed only one-fifth of the tissue, the inference was a sign of malignant characteristics in the villi. In these cases the sugar used as substrate was the sugar of the amniotic fluid as well as glucose added to Ringer's solution. Dickens and Greville (1932) showed that while all placentae caused anaerobic glycolysis of glucose, only three, those of the cat, ferret and man, would cause fructolysis at a lower grade when fructose was used. How their findings fit into the problem of the nature of placental activity is not clear, nor to what extent it has anything to do with carbohydrate metabolism. For this purpose more knowledge is required of intermediary metabolism of tissues.

The Metabolism and Transfusion of Lipids

As with carbohydrates so with fats, there are the two questions of transfusion across, and of storage and metabolism within, the placenta. This last question was the first to be studied in its comparative histology.

Fat is found in the extraembryonic tissues and, apart from that, in the allantoic placenta, in the amniotic fluid and in the yolk-sac.

In the amniotic fluid of the Ungulata are floating the hippomanes: in addition to the previously described glycogen, these also contain stainable fat (Jenkinson, 1913). In man, the fluid contains 0.20–1.22 per cent. of fat in colloidal solution (Prochownik, 1877, 1889).

In the yolk-sac it has mainly been observed in the rodents, rat and mouse, where the yolk-sac forms a definite placenta until the 11th day. After this the outer placental layer disappears, while the inner embryonic layer becomes highly villous. The tall columnar cells of this layer contain large basal droplets of fat. There is perivascular fat in the form of fine droplets to be found throughout the trophoblast, especially in the giant cells which in the rat replace the syncytiotrophoblast. The perivascular epithelial cells of the myometrium (endometrial gland) contain also a certain amount of fat. The yolk-sac entoderm has big droplets—the other cells have a fine dust cloud (Huggett and Pritchard, 1945a). Jenkinson (1913) regarded the yolk-sac in the mouse as acquiring fat by absorption from the uterine lumen which contains the fatty secretion of the uterine glands.

In the allantoic placenta, fat is stainable in certain definite sites in all species, though in varying degree in different types; these sites are the decidual cells, uterine epithelial cells and the leucocytes of the maternal portion of the placenta, the syncytiotrophoblast (giant cells in the rat), the cytotrophoblast and finally the endothelium of the villi. The outstanding feature in common to all species is the appearance of the fat in the maternal tissues, with varying erosion of these by the villi and the absorption by the trophoblastic villi of the fatty material (whether fatty degenerate debris or glandular secretion containing fat—"uterine milk" in the ruminant—Gamgee, 1864, Jenkinson, 1913) with the subsequent appearance of fat in the villi and later in the foetal viscera.

In the syndesmochorial placenta of the cow and sheep, fat is histologically demonstrable in the "uterine milk," and in the cells of the decidua, connective tissue and epithelium adjacent to the trophoblast cells, but only those which cover eroding villi.

In the endotheliochorial placenta of the Carnivora, there is practically no uterine secretion, and the leucocytes have considerably less fat.

In the rodent, after the vitelline circulation begins to atrophy at the 10th day, the haemochorial allantoic placenta develops. Fat appears in the decidual cells, and these are absorbed into the trophoblast. In the rabbit the decidual cells do not degenerate, but histologically degeneration occurs in these cells in the mouse, rat and guinea-pig, together with those of the surrounding connective tissue and endothelium, the whole forming a fatty debris. Simultaneously, fat appears in the giant cells of the trophoblast and the underlying cytotrophoblast.

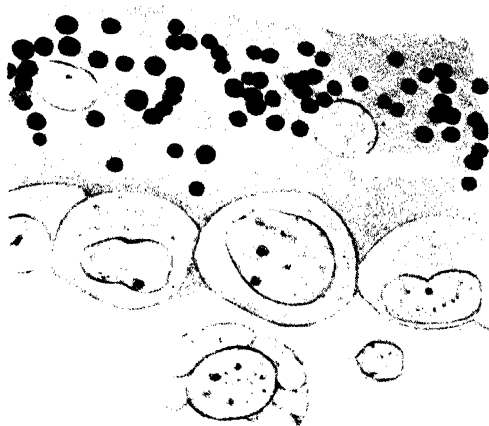


Fig. 16. 20—Human chorionic villus. 30 days. $\times 1600$. Fixed in Champy's fluid and unstained. Fat droplets in syncytium but absent from Langhans' layer, the Hofbauer cells, and from the stroma. (From Wislocki and Bennett, 1943.)



Fig. 16. 21—Human chorionic villus. 13 weeks. $\times 1600$. Fixed in Champy's fluid and unstained. Fat droplets smaller than at 30 days. In both cases there is often a halo around the individual fat droplets. (From Wislocki and Bennett, 1943.)

In man, with his haemochorial placenta, fat appears in the decidual cells near the villi at the second month, in the syncytium of the villi and also in the layer of Langhans (Apfelstadt and Aschoff, 1896), though many authors think that there is very little in the last layer (Wislocki and Bennett, 1943). When it appears in the stroma of the villi the minute droplets in the decidua are increasing though the deepest cells are showing fatty degeneration (Eden, 1897). Klein (1891) looked on this fat deposition in the decidua as being fatty infiltration in the early stages followed later by fatty degeneration. He regarded this last as being pathological; if, however, it occurs regularly and normally, fatty degeneration cannot be regarded as pathological, except in so far as normal senescence of the placenta is pathological.

The human syncytiotrophoblast appears to have a superficial fat-free layer and a deeper layer with stainable fat droplets which also appear in the mesoderm (Hofbauer, 1905). The syncytial fat is, according to Wislocki, stainable by fat stains, extractable with ether or acetone, shows birefringency when examined with polarised light, losing this after acetone extraction, often becoming crystalline and polarising light (common with steroids, rare with neutral fats); it may have a greenish fluorescence. Lastly, this syncytial fat forms a play of colours with concentrated sulphuric acid (as does cholesterol) and gives positive reactions for aldehydes and ketones with Schiff's leucofuchsin and by converting phenyl-hydrazine to hydrazones (Wislocki and Bennett, 1943; Dempsey and Wislocki, 1944), all of

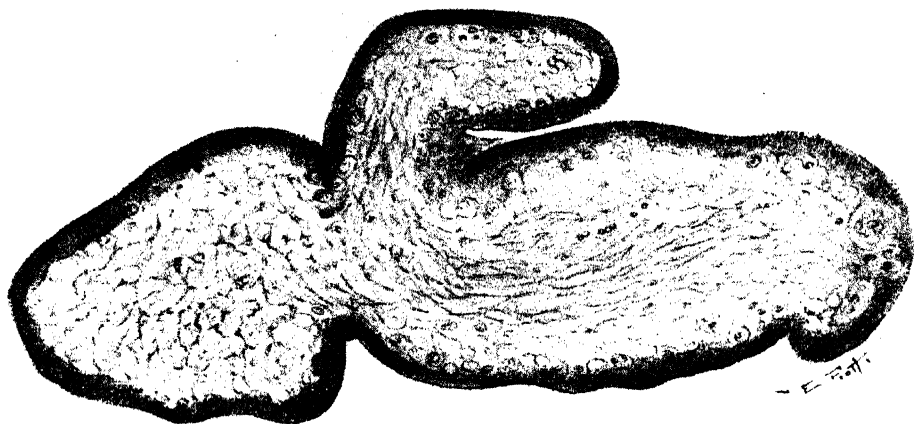


Fig. 16. 22—Human chorionic villus. 9 weeks. $\times 180$. Frozen and treated with phenylhydrazine to show the production of yellow phenylhydrazones. These are limited to the syncytium and indicate the localisation of the ketones and aldehydes. No yellow colour appears in control sections extracted with acetone and treated with phenylhydrazines. (From Wislocki and Bennett, 1943.)

which reactions disappear after acetone extraction. They suggest, therefore, that the syncytium of the placenta is the site of manufacture of the placental ketosteroids. In the cat, Wislocki and Dempsey (1946a) found the same location and came to the same conclusion, that the syncytiotrophoblast synthesises the placental hormones. In the pig, however, they (1946b) got such inconclusive staining reactions that they felt themselves unjustified in stating the sow's chorion synthesises ketosteroid hormones.

The biochemical picture adds more to the histological picture. The most striking point is the difference in different species. If the lipids in the blood, the plasma or the serum be observed during pregnancy, then there is a lipaemia in man (Slemons, 1919; Slemons and Stander, 1923; Boyd, 1934) but a lipopenia in the rabbit (Baumann and Holly, 1926; Popjak, 1946). These changes included neutral fats, phospholipins, cholesterol esters and free cholesterol;

the rise in this last was observed in man as early as 1913 by Klinkert when studying cholesterol changes in the blood in numerous conditions.

On comparing the lipid content of the maternal and foetal bloods or plasmas, the same species difference is observed. Kreidl and Donath (1910) found the foetal serum lipids exceeded the maternal in the guinea-pig; Klinkert observed excess of maternal blood cholesterol over foetal in man (1913); Slemons and Stander extended this to other lipids and showed, too, there was no appreciable difference in the toxæmias of pregnancy. Boyd (1934) demonstrated that this excess of maternal blood lipids extends to all fractions, but is almost entirely confined to the plasma, the red cells showing little or no change. Barcroft and Popjak (1945) found in the sheep (as in the guinea-pig) that there was an excess of maternal over foetal lipids (neutral fat, phospholipin, total fatty acids, cholesterol free and cholesterol combined). The foetal lipids followed the changes in maternal lipids which were rather irregular in the last days of pregnancy. Extending this further, Popjak (1946) showed that lipopenia in the rabbit is accompanied by an excess of foetal lipids (all fractions) over maternal plasma lipids. There is, therefore, the contrast between the two groups typified by man and rabbit, in the one a lipaemia accompanied by excess of maternal over foetal lipids, and in the rodent a lipopenia going with a deficiency of maternal lipids relative to the foetal lipids. These changes do not go with the Grosser's morphological types, since both the rabbit and man have haemochorial placentae.

Both Sato (1937) and Rosenbloom (1935) looked at the cholesterol content of the two bloods in the human, Sato finding a rising maternal and falling foetal blood cholesterol (umbilical vein and artery) as pregnancy progressed, while Rosenbloom recorded accumulations in the foetus, but with at full term the mother containing 223 mg. in her blood, while the foetus had only 120 mg., he was unable to decide whether the foetus was synthesising or storing it.

When fat is administered to a pregnant animal there is perhaps a rise in the maternal blood fat, but not of the foetal blood fat. This was first shown by Ahlfeld (1877) on feeding bacon fat to pigs and confirmed by Oshima in 1907, who was the first to use the blood chylomicron count for estimating fat, and showed, too, that fasting reduced the maternal, but not the foetal blood particle count. He decided that fat as such is not transported from the maternal to the foetal blood. In the human at full term, the maternal blood fat exceeds the foetal blood fat always (Slemons, 1919; Slemons and Stander, 1923).

But, if instead of examining the foetal blood fat, the foetal tissue fat be looked at, it becomes clear that either fat or fatty acids are not only traversing the placenta and reaching the tissues, but may be passing across almost unaltered. This has been demonstrated by giving the pregnant animal chemically identifiable fat, and examining the foetal tissues for it.

Hofbauer (1903) first labelled fat, using Sudan III and looked for it in the foetal fat after feeding it to pregnant guinea-pigs. He described it as passing to the embryo unaltered, the staining appearing in the foetal fat stores. This passage of dyestuff was not confirmed, however, by Gage and Gage (1909), nor other workers (Needham, 1931, p. 1192); from this it might be concluded that fat does not pass from the mother to the foetus across the placenta.

Hofbauer also labelled his fat by using freak fatty acids. Coconut oil contains two characteristic acids, myristic and laurinic. When Hofbauer gave coconut oil

to the guinea-pig he found laurinic acid in the foetal liver. From these results, conjoined with his Sudan III results (unconfirmed) he concluded that fat transfused the placenta as fatty acids and glycerol and after passing the superficial epithelium is resynthesised in the placenta and is transmitted to the foetal liver as glycerides.

About the same time the iodine value of the fat was used as a label, Thiemich (1905) determining that alteration of the dietary iodine value altered the maternal, but not the foetal, fat iodine value. This was confirmed by Wesson (1926) who, also using rats, showed that the foetal liver fat iodine value does follow the maternal iodine value which in turn follows the dietary iodine value. Sinclair also repeated Hofbauer's work with peculiar fatty acids in the dietary fats. He found that in rats, cod liver oil (1933) and trielaidin (McConnel and Sinclair, 1937) both traversed the rat's placenta to appear in the foetal liver and fat depots. Miura (1937) confirmed this and showed that elaidin, coconut and linseed oils all appeared in rat's foetal liver phospholipins.

It appears, therefore, that special fatty acids traverse the placenta, but there is no proof that unaltered glycerides pass through the placenta, and further stains combined with the fats appear to be completely stripped off them and retained by the placenta.

The lipide content of the human placenta is highest in early pregnancy and decreases later with placental progress; it is, however, exceptionally high in eclampsia (Bienenfeld, 1912). Quantitatively, this decrease applies to all ether-soluble materials after the 4th month (total lipids including, therefore, neutral fats, fatty acids and phospholipids), previous to that date they increase, to fall off after it until full term (Watanabe, 1923).

Boyd and Wilson (1935) assayed the lipids of the human umbilical cord at full term and showed the foetus was absorbing at the rate of 30 g. of phospholipin and 7 g. of free cholesterol per 24 hours. On clamping the umbilical vein, the blood in between the clamp and the placenta acquired a steadily rising content of lipids. They concluded that the fat arrived not by passive transfusion from the maternal blood, but by active thrusting by a dynamically active placenta. Boyd (1935) followed this up by estimating the neutral fat, phospholipins, cholesterol esters and free cholesterol in the placenta and foetus of the rabbit at different ages in pregnancy. The outstanding finding was that in the last part of pregnancy the neutral fat content of the foetus rose markedly in the last ten days of pregnancy. Simultaneously, there was an increase in the foetal phospholipins and free cholesterol. But in the placenta at this stage the outstanding change was a marked increase in phospholipins. Boyd attributes the foetal growth and neutral fat deposition in this last seven to ten days as being due to increased placental activity as signalled by the placental phospholipin rise. This he regards as inconsistent with a passive transference and more in keeping with a dynamic placental activity.

Effkemann (1936) studying infants at birth confirmed the results of Boyd and Wilson (1935). He demonstrated that the placenta transmits in 24 hours the amounts of fat found by Boyd and Wilson above (33 g. lecithin, 7.5 g. free cholesterol, 3 g. fatty acids and 8.5 g. of cholesterol esters), and that the maternal dietary fat can cause alterations by the foetal depot fat utilising preformed fat compounds. He also showed that the cholesterol esters are carried by the

serum, but the free cholesterol and phospholipin was in the cells. This led, therefore, to the problem of whether the foetal fat comes from the mother's fat (depots or diet), or from the carbohydrate supplies.

Chaikoff and Robinson (1933) showed that if pregnant rats were fed on a low fat diet, the foetal fat had iodine values in range of 72-74, whether carbohydrate or protein were in excess. If, however, the fat of the diet was increased in quantity, then the foetal fat acquired a big range of iodine value, proportionate to the dietary fat iodine value. The dietary fat altered the iodine value of the maternal depot fat over a range of 36 to 145 while the foetal iodine value ranged from 61 to 103. The inference from this is that the foetus can synthesise fat of iodine value 72 to 74 from maternal carbohydrate supplies, but if fat comes through to the foetus from the mother, it can be utilised to store as fat, influencing the iodine value of the fat so produced. In 1920, Imrie and Graham had studied the formation of fat in the liver of the foetal guinea-pig. They found its concentration steadily rose in foetal life. The iodine values were interesting. The iodine values of the maternal guinea-pig fats were, tissues (depots) 85, liver 120. In the foetus, the iodine value was 110 to 120, always well above that of the maternal tissues, suggesting that it came from the maternal liver. They now starved and phlorhizinised the pregnant guinea-pig. This gave still more fatty infiltration of the foetal liver. The new fat in the foetal liver had a low iodine value from which they inferred it was formed from maternal tissue fat, or from carbohydrate.

All this suggests, therefore, that both carbohydrate and fatty acids can leave the maternal blood and that the foetal and placental tissues can make fat from both sources, and that normally a preference is given in the foetal liver to maternal fat from the maternal phospholipins. This means that the foetal liver is deriving its fats from the same source (and with the same degree of freedom) as the maternal tissues get their intracellular fats, the desaturated phospholipins of the maternal liver.

Popjak (1946) tackled the problem experimentally from a new angle. He produced an experimental lipaemia in rabbits by feeding with amorphous cholesterol by the technique of Domosi and Egyed (1939). As a result, the normal plasma lipopenia of the pregnant rabbit was reduced in all fractions and restored nearer to the normal non-pregnant values (the free cholesterol was increased above the value for the non-pregnant adult). At the same time, the foetal plasma lipids which normally exceed the maternal were pushed still higher, and there was a big increase in the content of lipids in the placenta, particularly the foetal portion. All fractions were up, but especially cholesterol, free and ester. This increase was striking both to the naked eye and histologically. The appearance of the foetal portion was changed to a pasty yellow and there was a big increase in fat which was deposited in the endothelial cells of the foetal capillaries. Normally, as Chipman showed in 1903, the foetal placenta has practically no fat. The change was therefore very striking and recalls the atheromatous change in the virgin rabbits' arteries caused by feeding cholesterol, which Domosi and Egyed were studying in 1939. Accompanying this, the foetal weights dropped at the 28th day from an average of 32 g. to 22 g. There was clearly a pronounced deficiency in nutrition, which was supported by the fact that though there was the big increase in placental lipids, there was a big decrease in foetal lipids (except free cholesterol and phospholipids) especially neutral fats. The foeta,

decreases were almost entirely in the foetal livers. In contrast, the cholesterol diet had increased the lipids of the maternal livers. Popjak found, in fact, that the neutral fat content was not much impaired until the cholesterol content of the foetal placenta exceeded 1·8 per cent. when it decreased with the further rise in cholesterol content.

Popjak confirms Baumann and Holly's findings that the cholesterol and phospholipid phosphorus decreases in normal rabbit blood in pregnancy, but he extends it to all lipid fractions at the 28th day. In the normal pregnant rabbit the cholesterol and phospholipid decrease steadily, but the neutral fat shows a rise at the second week followed by a fall. This lipopenia is in the opposite way to the lipaemia of the human, it is not dependent on the number of foetuses and is not due to defective absorption, since ample reaches the foetus and placenta and it would appear to be due to a metabolic control by an unknown hormone.

Popjak considers that the foetal lipids are only partly derived from maternal fat and carbohydrate stores, citing the evidence earlier in this chapter. The rest of the foetal fat he considers to be produced actively by foetal or placental action, citing the experiments of Boyd (1934, 1935) and Boyd and Wilson (1935), and the results he obtained in the rabbit. To this he adds the experiments of Nielsen (1942) on rats. Nielsen fed pregnant rats with radioactive phosphorus and found the phospholipins traverse the placenta too slowly to account for the accumulation, and that, therefore, the phospholipins of the embryo must be synthesised after the placental barrier has been traversed.

The part played by lipase in the placenta needs to be considered. Kreidl and Donath (1910) had looked unsuccessfully for lipase in the guinea-pig's placenta. Anselmino and Hoffman (1929), however, did find it in the human placenta. They also found it had its maximum activity at the 4th month. This date is interesting in view of Watanabe's observation that the maximum concentration of ether-soluble material occurs at the 4th month also, though too much stress must not be laid on the mere conjunction of the two observations.

In connection with the carriage of phospholipins from mother to foetus across the placenta, four findings need to be considered together. Boyd found that increase in foetal phospholipin is associated with increased placental activity (active driving); Effkemann found that phospholipins are carried in the foetal blood cells; Popjak found that feeding cholesterol increases the phospholipin in the maternal portion of the rabbit's placenta and decreases it in the foetus, and Nielsen (1942) demonstrated with radioactive phosphorus that phospholipins traverse so slowly across the placenta, that possibly they are synthesised in the embryo from small molecules which have effected the actual passage. It would appear, therefore, that while a gradient for the passage of phospholipins may exist, the passage is extremely slow and governed by at least two important factors, namely, placental activity and the cholesterol content of the diet not to mention the transport by the blood cells.

In summing up, two things need to be borne in mind: neither Hofbauer's nor Sinclair's results really show how the fat actually traverses the placenta—they neither prove nor disprove the hydrolysis at the placental membrane and subsequent resynthesis in the deep syncytium. All they show is that particular or labelled fatty acids originally on the maternal side appear later in the foetal tissues.

Further, the presence of lipase in placental extracts does not prove that it effects this hydrolysis, any more than that its presence in intestinal villi means that fat cannot pass from the lumen of the intestine to the portal blood in molecular unhydrolysed form. Needham (1931, p. 1479) reviewed this problem, and also concluded that while there was evidence of placental lipase, there was no evidence that this lipase ever caused the placenta to synthesise fat from glycerol and fatty acids, but that the reverse even actually occurred.

To this may be added that the apparent force existent in the placenta, which Boyd describes driving fat into the umbilical vein, does not necessarily mean a lipase is synthesising fats from fatty acids and glycerine. There is at the moment no evidence to enlighten us as to its nature. It is a phenomenon worthy of confirmation and further study. In connection with this question, while fat appears in the syncytium it is not necessarily fat in transmission from the mother to the foetus, as Hofbauer thought, but, rather, in view of the findings of Wislocki and his colleagues, and of Boyd and Popjak possibly metabolic fat produced in the placenta during the intracellular metabolism. Wislocki gives good reasons for this (outlined above), suggesting that it is the seat of formation of the placental steroid hormones.

The Transmission of Vitamins across the Placenta

The fat-soluble vitamins

These behave differently from the water-soluble vitamins in that the relative solubility appears to enter into the problem. They all appear to be held up on the placenta. This was demonstrated for vitamin E by Mason and Bryan in 1940, and for vitamin D in the human placenta by Guggisberg (1929) where large quantities appear to concentrate. With vitamin A its passage is impeded at the placenta, so that the foetal liver at term contains much less than the adult liver (Wolff, 1929; rat and rabbit—Dann, 1932) in all placental types studied. Dann carried this further showing the new-born calf only has 600 B.U. of vitamin A in its body, but acquires 55,000 B.U. in its colostrum after birth; he further showed in the rat that the amount transfusing was barely raised if the dietary content was elevated, but increased ten-fold by a twenty-fold increase in maternal dietary fat—that is, increased fat increased the amount of vitamin A delivered to the foetus. Neuweiler (1936a), reverting to human beings, thought there was a higher concentration in the foetal liver in early pregnancy than in late pregnancy, while Wendt and his colleagues (1936) in a systematic examination of 40 human cases at varying gestation ages concluded that there was, (i) no vitamin A and little carotene in the placenta; (ii) only a little vitamin A in the foetal blood, though considerable quantities might appear in the maternal blood; (iii) vitamin A in the foetal liver, though less than in the maternal liver, and (iv) a higher concentration in early foetal life than at term (confirming Neuweiler, 1936a). Wendt concluded that the placenta does not actually store vitamin A, but does impede its transmission, a conclusion fitting in both with Dann's results on animals and the findings in regard to other fat soluble vitamins. Gaehtgens (1937a) found it both in the full term foetal liver and in the placenta (1937b), which he regarded as a store for it. Contradictory results obtained by other workers could be all explained by variations in the maternal dietary fat to which no attention was paid.

The water-soluble vitamins

There is a considerable body of literature relating the incidence of neuritic pains in human pregnancy to a deficiency of vitamin B₁ in the diet. This is summarised by Nixon, Wright, and Fieller (1942). These authors, in addition, show that in eclampsia the excretion of aneurin in the urine diminished (0.94 I.U. per day) compared with normal (3.39 I.U. per 24 hours). Further they demonstrated that the normal placenta contained 19.4 I.U. per 100 g., while in eclampsia there were only 5.9 I.U. per 100 g. of placenta. These interesting figures must be accepted only for what they are, since in toxæmias, other than eclampsia, there were equally striking figures, increases in oedema of pregnancy (32.3 I.U. per 100 g.) and pre-eclamptic toxæmia 21.3 I.U. per 100 g.). Since practically nothing is known of the lesions and the causes of the toxæmias, it has to be stated clearly one must not make too sweeping deductions as to their meanings.

In regard to vitamin C (ascorbic acid) there is evidence that it is stored in the placenta and decidua (Neuweiler, 1935, 1936b) and that the quantity varies with the maternal diet. It appears in double the concentration in the blood of the human umbilical vein than in that of the umbilical artery (Neuweiler, 1935), suggesting that it passes freely from the placenta to the foetal blood, and is actively removed by the foetal tissues. Wahren and Rundquist (1937) showed that it was normally at a higher concentration in the foetal than maternal blood, and both values are raised by orange juice (Monahan and Eastman, 1938; Fleming and Sanford, 1938). There is considerable storage of ascorbic acid in the suprarenal gland of the foetus of the rat and guinea-pig (52–132 mg. per 100 g.); higher values than in the adult (41–80 mg. per 100 g.).

The Transmission of Nitrogen

This function of the placenta can be considered chronologically under two headings, the early period when the chorionic villi are invading the decidua (in varying degree according to the species), and later when the placenta is being traversed by diffusible materials from the maternal to the foetal blood and vice versa.

The early period

The absorption of protein in the early period is bound up with the absorption of the embryotrophe by the trophoblast.

In all types there is evidence that haemoglobin and debris of cells—decidual, epithelial and blood—can be taken up phagocytically, the degree varying with the different types. In the sheep for example, colloids (fat globules) are present, but active engulfment of cells is not visible (Assheton, 1906), whereas in the cat (Duval, 1893) blood cell remains are demonstrable. In the primate (Wislocki and Bennett, 1943) the matrix of the cytotrophoblast contains protein from different sources, but mainly from the decidua and maternal blood.

The mode of entry of unhydrolysed molecular proteins and particulate bodies into the cell's is unsettled. It is considered with the total evidence on transfusion of colloidal and organised bodies.

The later period

If the transfusion of the diffusible nitrogenous compounds be examined in the light of their relative concentrations on either side of the placenta, the data are summarised in Table VIII.

TABLE VIII

CONCENTRATION OF NON-PROTEIN NITROGEN AND AMINO-ACIDS (MG. P.C.) IN THE FOETAL AND MATERNAL BLOODS

Animal.	Substance.	Maternal Blood.	Foetal Blood.	Remarks.
Man	N.P.N.	17·8	18·7	Morel and Mouriquand, 1913
	Amino Acids	5·6	6·0	
Rabbit	N.P.N.	29·0	29·0	Snyder and Hoskins, 1929
	A.A.	9·0	9·3	
	N.P.N.	34·0	43·0	Slemons and Morriss, 1916, 1917 Whole blood } Collip, 1927 plasma }
Dog	N.P.N.	40·0	41·4	
Cow	N.P.N.	28·9	48·4	
	N.P.N.	28·4	38·8	

From those figures alone it appears that in almost every case there is an opposition gradient to the flow of diffusible materials from mother to foetus.

Buglia (1913) carried the problem further and in the dog altered the level of the non-protein nitrogen in the maternal blood (Table IX). The protein hydro-

TABLE IX

NON-PROTEIN NITROGEN IN MG. P.C.

	Maternal Blood.	Foetal Blood.	Maternal Urine.	Amn. Fluid.	Allant. Liq.
Normal	45·0	56·0	716·0	58·0	159·0
After saline (Control)	48·0	64·0	—	—	
After protein hydrolysate injection	110·0	91·0	271·0	85·0	222·0

lysate injected as non-protein nitrogen was made by hydrolysing Witte's peptone with trypsin. This experiment shows that elevation of the maternal blood N.P.N. raised the N.P.N. concentration on the other side of the placenta, but it does not rise so much as in the maternal blood; it apparently transfuses, but is slow enough to suggest that the elevation has been accomplished by simple diffusion; the gradient achieved allows for this.

Luck and Engel (1929) approached the question differently. They injected glycine and alanine subcutaneously into the pregnant rat, and estimated the amino-nitrogen content of the maternal liver, maternal muscle and foetus at regular intervals after injection. The uptake of the amino-acids by the placenta and foetus was much less than that of the liver and nearly as high in altitude and shape as the uptake by the maternal muscle. That the liver has a particular ability to abstract amino acids from the circulating blood is well known.

They inferred that the mechanisms of absorption of amino-acids by the placenta and by maternal muscle were identical. It could be a question of diffusion and could occur in this manner despite the opposition gradient if there was evidence that there was a Donnan equilibrium set up across the placental membrane. On this, however, there is no further evidence forthcoming. In other words, a negative gradient need not rule out diffusion of amino-acids from maternal and foetal bloods.

The passage of the colloidal proteins opens up two possibilities, hydrolysis by enzymes, such as proteases and the passage of unhydrolysed proteins. It has already been shown that lipides and fatty acids can pass across the placenta and there is no conclusive proof that the fats are first hydrolysed, though lipases are detectable, but rather is there circumstantial evidence that they may in many cases transfuse in the molecular unhydrolysed form.

Many intra-cellular proteases have been demonstrated in almost all species of placenta examined. Glycerine extracts of the placentae of the sheep and rabbit show no proteolytic enzyme in the sheep ; but in both the maternal and foetal portions of the rabbit's placenta crepsin has been identified (Lochhead, 1908). In the human placenta almost every type of protease has been found, peptic, tryptic and ereptic enzymes by Maeda (1923) and also cathepsin (normally found as an intra-cellular enzyme in normal tissues). This latter has been studied by Goldstein and Milgrom (1935) who show that it exists in two forms in the placenta, activated and unactivated and that synthesis of protein is proportional to the excess of the former over the latter. In the rodent placenta, synthesis is more rapid than in the human placenta. The protease of the human chorionic villi is maximal in the first three months of pregnancy but decreased in the fourth month and then disappeared (Graefenberg, 1909a and b).

As with lipases, so with the proteases, their mere presence is no evidence of their normal task and does not prove that they are concerned with the passage or metabolism of proteins in the placenta. But there is considerable evidence of lipid synthesis (Boyd, 1935 ; Popjak, 1946) and it is highly possible that protein synthesis can occur.

Dempsey and Wislocki (1945) assembled the evidence that the ribonucleo-protein content of tissues is detectable by basophilic staining which was destroyed by digestion with crystalline ribonuclease. They showed this to be the case with the basophilic reactions of the syncytium and the cytotrophoblast of the human placenta (Dempsey and Wislocki, 1945). In the cat's placenta, Wislocki and Dempsey (1946a) found (as in the human) that in the first half of pregnancy there is a conspicuous amount of ribonucleoprotein which disappears in the second half and is replaced by alkaline phosphatase. This finding for cats and humans is also true for rodents, but not for the pig (Wislocki and Dempsey, 1946b), where the basophilia lasts throughout pregnancy in the trophoblast and alkaline

phosphatase only appears near the brush border of the chorionic fossae cells. They suggest the alkaline phosphatase causes hydrolysis of the ribonucleoproteins. They regard the ribonucleoproteins as being proteolytic enzymes engaged in protein and hormone synthesis. In the uterine glands, basophilia is maximal in the pig (where uterine milk containing protein and Schiff-positive material is secreted), less in the dog and least in the human where there is little glandular activity, the protein foodstuffs for the human embryo coming from the maternal blood (Dempsey and Wislocki, 1946).

The unhydrolysed proteins

In the case of unhydrolysed proteins, there is considerable evidence that they can traverse placenta in trace quantities, detectable by immunological methods, but little or no evidence for the passage in quantity sufficient for the protein deposition of growth.

The epitheliochorial placenta of the horse (Polson, 1943) and the syndesmo-chorial placenta of the ungulate are impermeable to antitoxins (Mason, Dalling and Gordon, 1930). It is of interest to note that the calf acquires its antibodies from its mother in the colostrum (Orcutt and Howe, 1922 ; Jameson *et al.*, 1942). The haemochorial placenta of the rabbit is, however, permeable (Ratner, Jackson and Gruehl, 1927). This permeability increases from the 22nd to the 30th day (full-term), at any rate for agglutinins and haemolysis (Rodolfo, 1934), during which time the placenta alters from a haemomesial to a haemoendothelial placenta. But not all proteins will pass across the rabbit's placenta ; Lochhead (1908) injected foreign proteins (adopting Ascoli's 1902 technique) into the mother and tested for it against immune rabbit serum. He found egg albumen passed across, but not the serum proteins of the ox. In the human, where there is also a haemochorial placenta, there is evidence of differential passage of certain agglutinins and antibodies.

There is considerable evidence that there is a relative deficiency of globulin. In 1918, Alder, comparing the protein content of maternal and cord blood, found the maternal averaged 7·8 per cent., of which 2·6 per cent. was globulin, while the cord blood averaged 5·7 per cent., of which 1·6 per cent. was globulin. In the premature infant there is 4·94 per cent. protein, of which 1·18 is globulin ; at full term this has risen to 5·52, containing 1·78 per cent. globulin, and at 5-6 months it has risen to 6·29 per cent., containing 2·14 per cent. (Darrow and Cary, 1933). Longsworth and his colleagues (1945) have shown that at birth, however, the full-term human foetal serum has relatively more γ -globulin but less α and β -globulin, and less albumen than adult serum. Marrack (1946) suggests that the serum of premature infants may contain less γ -globulin than that of adults.

On the other hand, while there appear to be adequate supplies of γ -globulin, but not of the other protein fractions, there is evidence that equilibration of diphtheria antitoxin occurs across the placenta (Charrin, 1898 ; Liebling and Schmitz, 1943).

In 1941, the whole question of the permeability of the placenta to agglutinins increased in importance when Levine, Katzin and Burnham demonstrated a new aetiology for erythroblastosis foetalis. This is a condition in which the infant dies in utero or shortly after birth, showing signs of destruction of erythrocytes, anaemia, jaundice and immature red cell formation, accompanied by signs of

over-activity of the bone marrow. They showed that the foetus in utero inherits an agglutinin from its father, which is absent in the mother. This agglutinin (called Rh) is one normally in Rhesus monkeys and in 85 per cent. of humans. It can apparently transfuse the placenta from the foetal to the maternal blood, and immunise the mother and form isoagglutinins for Rh, which then go back to the foetus and, reacting with the Rh agglutinogens, destroy the cells and cause a secondary erythroblastosis, the whole proceeding to such a degree as to be fatal. The Rh factor is carried as a Mendelian dominant (Landsteiner and Wiener, 1941). Now Haldane (1942) has shown that, allowing for random mating, if the mother is *rh rh*, and the baby having an Rh factor from its father is *Rh rh*, then babies developing the disease form only 5 per cent. of the number that should do so on genetic grounds alone. Further, half of these mothers are sibs. Ten per cent. of cases of erythroblastosis foetalis are due to anti-A or anti-B agglutinins from A or B negative mothers going to embryos with A, B or AB agglutinogens. Again, of these, the actual number is half the expectation (Boorman, Dodd and Mollison, 1944). Many factors and explanations have been put forward. One such factor might be variations in molecular weights of the antigens or of the antibodies. The A factor has been subdivided into A_1 and A_2 ; the Rh into 8 types by Fisher (Race, 1944). There is evidence of difference in solubility of the antibodies, anti-A and anti-B, but such evidence is not known with certainty for anti-Rh. Haldane visualises it rather as a biological variation in permeability in different placentae. Biologically variable permeability of placentae within the species is quite possible. Dienst (1905) perfused 160 placentas of women at childbirth with methylene blue through the umbilical vessels, and showed that the dye only appeared in the maternal urine in 20 per cent. of cases.

Tovey (1945) has confirmed the differential permeability hypothesis of Haldane; he has also suggested that the placental barrier is greater for anti-A or anti-B than for anti-Rh factors, hence the 90 per cent. incidence of the Rhesus type of erythroblastosis foetalis. Still further, he has shown that foetal bloods may contain group specific substances which neutralise the incoming agglutinins.

This concept of an agglutinin from the foetus immunising the mother and the maternal agglutinin returning to the foetus across the placenta is quite clearly extendable to other naturally occurring proteins. It hinges on the differential increase in the permeability of the placenta in late pregnancy.

IV. PLACENTAL EXCRETION

The placenta transmits waste products from the foetus to the mother. Savory first demonstrated in 1858 that materials could pass to the mother from the foetus by injecting strychnine into two out of four embryos of a pregnant bitch, when he found these two and their mother died within nine minutes, but that the uninjected embryos survived. In the rabbit the mother died before the embryos when the same experiment was repeated. Preyer (1887) got similar results using nicotine, hydrocyanic acid and curare. In 1898 Charrin showed that diphtheria toxin passes from mother to foetus. Preyer showed the transmission of carbon dioxide in the sheep, and Huggett in 1927 showed this was dependent upon a positive diffusion gradient from foetus to mother and reversed with reversal of the pressures.

Considering the normal nitrogenous components of the urine, the urea appears to be practically identical in the two bloods in almost all species (rabbit—Morel and Mouriquand, 1913; Snyder and Hoskins, 1929; man and dog—Slemons and Morriss, 1916). Intravenous injection of urea in the pregnant rabbit (Brandstrup, 1929) causes a parallel rise in the foetal blood urea. In man, the urea (plus ammonia) is the same in the maternal blood (10.5 mg. per cent.) as in the blood of the umbilical artery (10.4 mg. per cent.) but if the mother for pathological reasons has a high blood urea, then the foetal blood urea is correspondingly elevated (Slemons and Morriss, 1919).

The total creatinine is always higher in the foetal than in the maternal blood (Hunter and Campbell, 1912; Plass, 1917). The earlier workers found 1.67 mg. per cent. in the maternal and 1.75 mg. in the foetal blood, while Plass found 1.70 mg. and 1.75 mg. per cent. respectively. Uric acid, like urea, is identical, too, in man (Slemons and Bogert, 1917) at 1.5 mg. per cent. Put briefly, there appears to be complete permeability of the human placenta with full equilibration for urea, uric acid and creatinine and also for urea in the dog and the rabbit.

The mechanism of passage across the placenta has been considered. In the primates and the rodents the syncytiotrophoblast is thinned out to thin plates over the vessels of the chorionic villi. Bremer (1916) studied the interrelations of the placenta with the mesonephros and the metanephros in different types of animals. He pointed out that in the *Ungulata* and *Carnivora* this thinning of the syncytiotrophoblast does not take place and the rate of elimination appears to be slow throughout most of foetal life, whereas in the rodent, excretion is wholly through the placenta in the rat and in the rabbit and guinea-pig, first through the Wolffian body and later through the placenta. In primates (man) the excretion is as in the rabbit and guinea-pig. He therefore compares the thin syncytial plates with the flattened endothelium of the capsule of Bowman in the adult kidney. However, Wislocki and Bennett (1943) reinvestigated the histology of the primate placenta from the functional viewpoint and showed it contains a few lipoidal droplets, a stubble-like surface and some mitochondria. The latter, usually regarded as significant of enzymatic activity, can be regarded in this case as a sign that though the plates of syncytium are thin, they are not passive and inert material for mere diffusion. Gersh (1937) has studied the functional activity of the mesonephros and shown there is cytological and analogical evidence of activity. The excretion of phenol red and ferrocyanide was effected slowly through the mesonephros in the opossum while living in the pouch, and also slowly in the foetuses of the cat, dog and rabbit (which he attributes to the low foetal blood pressure).

V. PLACENTAL PERMEABILITY

There are four classes of materials from which evidence may be gathered as to the manner of placental functioning in the transfusion of materials across it. These four classes include:

1. The bacteria and viruses.
2. Dyestuffs, both colloidal and crystalloidal.
3. Unhydrolysed organic colloidal molecules such as fats and proteins.
4. Gases and diffusible crystalloids including hydrolysates.

The Passage of Bacteria, Viruses and Solids

Treponema pallidum is never recovered from the embryo before the 24th week (Harris, 1929), previous to which senescent changes are not present in the placenta, or the 5th month (Hoffman, 1936); there is no evidence of its reaching the embryo except across the placenta (Hoffman). It was first shown in 1902 (Neelov) that organisms normally cannot pass a healthy placenta, and that pathogenic ones only traverse it after producing lesions. This was confirmed in the rabbit by Nakagawa (1936) who also showed that the passage of the pathogenic group is accelerated by toxins, but impeded by adrenalin injections, which presumably act by restricting the maternal blood supply to the placenta. Infants have been born with erysipelas. This is due to a streptococcal infection. There is no evidence as to the age of the youngest known embryo so infected, nor, therefore, at what placental age the organism reaches the foetus, nor of the placental condition.

The viruses and phages can barely be called particulates or organisms: in any case, the evidence is contradictory. Bacteriophage against *B. coli*, *typhosus* and *dysenteria* (Flexner) have been stated to traverse the guinea-pig's placenta at all ages (Blair and Reeves, 1928), but this has been contradicted by Grasset (1927), who has been confirmed by Natan-Larrier, Eliava and Richard (1931). On the other hand, rabies virus can pass the placenta in dogs and rabbits (Lanfranchi and Lenzi, 1918). It should also be noted that infants have been recorded as being born with rashes of scarlet fever, small-pox (virus diseases) and also with pemphigus a disease of unknown origin, possibly a virus.

It is of importance to note here the occurrence of congenital defects of the heart and cataract of the lens of the eye in infants born of women who are infected with German Measles (Rubella) in early pregnancy, the 5th to 8th week of intrauterine life (Gregg, 1941; Swan, 1944). It is not described when the infection is later in pregnancy. The virus therefore passes the placenta at this early age, which is about the date when the allantoic mesodermal circulation fuses with the ectodermal trophoblast to form a complete foetal placenta. It is also the age when the septa of the heart are forming and the bulbus cordis is evolving. It is possible to look on the virus as impairing the enzyme systems involved in lens and heart formation.

This passage in early foetal life links with the observations of Warkany and his colleagues that foetal skeletal deformities can be produced by riboflavin deficiency in the pregnant rat, provided it is produced before the 13th day. The 11th day is a critical date in placental history, it is the date when the allantoic mesoderm join the ectoderm (Warkany and Nelson, 1941; Warkany, Nelson and Schraffenberger, 1942, 1943; Warkany, 1945). The point is reconsidered on page 381 in a consideration of this critical date.

It appears, therefore, that all placentae are relatively impermeable in early placental life to organisms, but become more permeable normally in later placental life with the disappearance of some of the placental layers, so much so that even large particulate organisms such as *T. pallidum* will traverse the placenta. This same increase in permeability is achieved, too, by disease and damage of the placenta, such that pathogenic organisms to which the placenta is in health impermeable can pass across after causing pathological changes.

Considered therefore in terms of some known sizes we obtain the data in Table X.

TABLE X
ORGANISMS, VIRUSES, PHAGES AND PROTEIN MOLECULES
Diameters in millimicrons
(After Laidlaw, 1938 and Elford, 1938)

Name.	Diameter in $m\mu$.
Streptococcus)	
Staphylococcus)	1000·0
Bacillus prodigiosus	750·0
Psittacosis virus	250·0
Spirochaeta pallidum	200·0
Vaccinia virus	150·0
Rabies virus	125·0
Bacteriophage staph. aureus	75·0
„ typhosus 105	65·0
„ coti " WLL "	60·0
„ dysenteriae (Flexner)	46·0
„ typhosus T III	25·0
Haemocyanin	24·0
Bacteriophage S 13)	
Poliomyelitis virus)	10·0
Edestin	8·0
Serum globulin	6·5
Serum albumen)	
Oxyhaemoglobin)	5·0
Egg albumen	4·0
Range of proteins	4·0- 24·0
„ „ phages	10·0- 65·0
„ „ viruses	10·0- 250·0
„ „ organisms	150·0-1000·0

The passage of immunological quantities of proteins, as described in earlier parts of this chapter, (e.g. Rhesus factor), is seen therefore to be the passage of molecules of the general size of 4·6·5 $m\mu$. Vaccinia and rabies viruses, both of which can traverse the placenta, are of the order of 125-150 $m\mu$ diameter. *Treponema pallidum* is a relatively small organism of 200 $m\mu$ diameter compared with, at the other extreme, the cocci of 1,000 $m\mu$ diameter. But as has been said, we have little evidence that any of these substances or organisms can pass across an unatrophied placenta, nor have we any information about the relative permeability of different layers in different species to this group, nor the age and condition of the placenta when it does become permeable.

The passage of solid particles, such as organisms, from the maternal blood to the foetal blood, or from the maternal tissues to the foetal tissues is, so far as the limited evidence goes (the passage of *Treponema pallidum* after the 5th month), quite different from phagocytosis of visible particles by the trophoblast. This is a function which appears to be lost in the early stages of placental development when the blastocyst has completed its invasion of the decidua (*see* the earlier part

of this chapter on the passage of iron). Indian ink is not absorbed by the rabbit's trophoblast (Wislocki, 1921) and all the cases of red blood cells, leucocytes, uterine epithelial cells, decidual tissue, etc., described as being absorbed, are always in the early stages of ovum development and confined to the peripheral trophoblast (Wislocki and Bennett, 1943). But it is clear that fluids with large molecules in solution are absorbed with relative ease and it has been pointed out by Hill (1932) that much material tissue that is absorbed undergoes extensive cytolysis.

The Passage of Dyestuffs

As has been said in the section on fat transfusion, Hofbauer (1903, 1905) found Sudan III dissolved in fats went with the lipids into the foetus. This passage of dyestuff was not confirmed by other workers. On the other hand, if a solution of methylene blue be perfused into the human umbilical cord at birth, it appears in the urine of the mother in 20 per cent. of cases (Dienst, 1905). Methylene blue has not only a smaller molecule weight than Sudan III, 282 against 371, but it is highly diffusible; both give true solutions, non-colloidal, Sudan III differing only in its high solubility in fats. Dienst's results suggest a differential permeability of the placenta in different individuals (J. B. S. Haldane's explanation, 1942), but this view is open to the objection that placental separation or blood clots may have occurred in 80 per cent. of the cases. Nevertheless, the diffusible dyestuff can traverse easily from the foetal side in a number of cases.

When trypan blue (mol. wt. 990) was injected intravenously in mice, to study its uptake by tissues, both normal and malignant (Goldmann, 1909), it was found to diffuse into the decidual cells, the amniotic fluid, the ectodermal (trophoblast) cells of the villi, but it did not penetrate beyond the chorionic epithelium into the sub-ectodermal mesoderm nor into the foetus. If, however, it is injected into the amniotic fluid of the cat (Wislocki, 1920) or the guinea-pig (Wislocki, 1921a) it is picked up by the foetus, but again never passes back across the chorionic epithelium. Conversely, if it is put into the mother, Wislocki found, as Goldmann did, that it does not penetrate, nor reach the foetus (Wislocki, 1921b). This latter observation correlates possibly with the atrophy of the rabbit's placenta to a haemo-endothelial type at full term. On the other hand, the vitelline cells (entoderm) store trypan blue quickly and avidly except where covered by ectoplacental ectoderm. This difference between the cat and the rabbit centring on the presence or absence of the chorionic epithelium is shown by Cunningham's (1920, 1922) observation that sodium ferrocyanide passes from the mother to the foetus in both species, but iron ammonium citrate only in the rabbit (where the trophoblast atrophies), but not in the cat, where it persists. Both dyes pass the entodermal yolk-sac placenta in the rat at the 9th day, but the ectoplacental cone was impermeable (Brunschwig, 1927). Similarly, toluidine blue (mol. wt. 315) traverses the yolk-sac epithelium more easily than it crosses the trophoblast of the allantoic placenta, which is histologically thinner. But toluidine blue did pass the trophoblast to some extent whereas trypan blue (mol. wt. 990) did not traverse it all, though it entered into the cells of the epithelium (Everett, 1925). This was reminiscent of Cunningham's distinction in the trophoblast between its ability (a) to pick up dyes, (b) to store dyestuffs, and (c) to pass dyestuffs on to the foetus. In 1920, Shimidzu, in a systematic study, tried to classify dyestuffs by

their passage across the placenta and to classify placentae by this permeability to dyestuffs. He showed that, with a few unexplained exceptions, diffusibility in the placenta was proportionate to the dialysability of the dye and, further, that if he studied the diffusibility across gelatin membranes of different concentrations, there was (again with few exceptions) parallelism between the permeability of the placenta and the diffusibility in gelatin of 25 per cent. concentration.

The emphasis on the molecular weight of a dyestuff which is made above is, it should be pointed out, only on one important property. A thorough consideration should take into account many other properties both chemical and physical. Such would include basic and acidic groupings, solubility in water and fat solvents, influence upon physical properties of membranes and solutions. These points have not been approached in placental physiology.

Anselmino (1929) followed up Shimidzu's line of attack on the problem by using solutions of biological substances instead of dyestuffs. He selected materials which were non-electrolytes, insoluble in lipids, had no particular surface tension properties, and were easily estimated (e.g. urea, sugars). He found that if the molecular weight was less than 350, diffusion alone seemed to be adequate to explain the passage. Above that weight other actions came into play.

The primate placental trophoblast has the power of ingesting microscopic particles of dyestuffs, e.g. Indian ink from the maternal blood stream, but it is restricted to the early stages of gestation—it is gone by the 58th day in the Rhesus monkey (Wislocki and Bennett, 1943).

From these experiments on dyestuffs it appears that they will diffuse with ease when of low molecular weight and freely dialysable, but in those cases where the molecule is relatively large, over 350 or more, other factors enter the picture, one of which may be solubility in placental lipids. These tend to inhibit the rate of diffusion. Further, the layer which exerts most selective influence is the ectodermal trophoblast. Lastly, the trophoblast has even greater powers of selection in regard to solids of microscopic size, in that in early placental life in the rodent and in the primate it can phagocytose substances such as carbon particles or formed elements, but not in later life. On the other hand, in the ungulate placenta this phagocytic power extends apparently throughout the greater part of pregnancy (Assheton, 1906), if not the whole period. This is also associated with an opposing gradient to sugar diffusion in these placentas. Correspondingly in the rodent and primate the diffusion of materials is accelerated in later life, long before which all phagocytic power by the placenta appears to have gone.

The Permeability to Organic Colloids

Lipid transfusion

The one thing which is clear about the transfusion of lipids is that it is a complex biological process, differing probably for the four main types, neutral fats, phospholipins, cholesterol and cholesterol esters. Lipids pass across with a gradient, apparently driven by a force generated in the placenta; there are decidua deposits and trophoblastic deposits. Cholesterol feeding causes a big increase in the foetal deposits of all types of lipids in the placenta, but localised mainly in the endothelium of the foetal vessels. This deposition is accompanied by a loss of foetal weight and a diminution in the lipid deposits in the foetus

and its liver. There is no reason to consider fat transfusion across the placenta as being any simpler than it is from the intestine, where it goes partly hydrolysed as fatty acids and glycerol and partly in molecular form and where the molecular transfusion is modified not only by cholesterol but also by bile salts, by phospholipins and by choline.

Protein transfusion.

There is the probability that the mass of protein for purposes of nutrition is transfused as amino acids, despite the fact that there is an opposition gradient. The experimental results show approximate equilibration between the two bloods. Similarly for the transfusion of nitrogenous waste products from the foetus. There is some transfusion of unhydrolysed molecular proteins (of diameters up to $6.5-9 \mu$) from the mother, but this is only in quantities of immunological importance and is valueless for nutritional requirements. The same phenomenon is present in the adult in varying degree in different sites (intestine, respiratory tract, skin) being most evident in allergic cases.

As has been said, Lochhead (1908) showed that the rabbit's placenta is permeable to egg albumen, but not to serum protein of the ox. He was interested in the placental proteases and suggested that ox proteins were more easily metabolised than egg albumen, which therefore passed unaltered. He thought this was abnormal and that rabbit maternal serum proteins could not pass, but normally all proteins are first hydrolysed to smaller units. In the guinea-pig, lactalbumen and the serum proteins of the horse fail to pass, but egg albumen (as in the rabbit) does pass, as also antibodies of all these three types of proteins (Natan-Larrier and Grimard-Richard, 1932). On the other hand, egg albumen has the peculiar property of not only crossing the placenta itself but of enabling horse serum proteins, which alone will not cross, of passing over themselves (Natan-Larrier, Noyer and Richard, 1931). This latter property egg-albumen shares with bile salts (Natan-Larrier, Richard and Noyer, 1930). The mechanism of this adjuvant action is unknown—it is not produced by lowering the surface tension of the maternal serum (Natan-Larrier and Grimard-Richard, 1933).

The ability of the placenta to absorb immunological quantities of proteins from the maternal blood is not quite the same as that of the trophoblast to ingest proteins such as haemoglobin or whole cells or other particulate matter. This latter quality is the essence of the phagocytotic action of the chorionic villi when the blastocyst erodes the maternal tissues.

The passage of gases

As described earlier in this chapter and in Chapter 17, oxygen and carbon dioxide appear to pass across by diffusion. This permeability to oxygen and carbon dioxide is, however, not so full as that of adult capillaries. There is an adequate gradient: the quantity traversing is diminished by lowering the tensions and apparently reversed by reversal of the gradient. The rate of equilibration is slow compared with equilibration rates in adult lungs or between capillaries and adult tissues. The speed of equilibration is helped by the morphological arrangements of the blood vessels of the mother and foetus in the placenta, but the speed is impaired by the competing metabolism, resulting in a pronounced diminution of oxygen saturation in the blood of the uterine vein. This is due in part to the

considerable amount of tissue between the two (maternal and foetal) blood streams in which the evidence shows there is an active metabolism of foodstuffs, to mention only fat and glycogen. It is of interest to note that in the human placenta, coincident with the maximum loss of weight at full term, there is not only greatest approximation of the blood streams, but also a decrease in the oxygen metabolism (Wang and Hellman, 1943).

The transfusion of the diffusible inorganic constituents

This is by no means one of simple diffusion. Table II shows how the haemochorial villous type of placenta in man has passing across it components of the blood streams, some of which go apparently against the gradient, some have a helping gradient and some apparently are in such close concentration on both sides of the placenta that normally they can be looked on as equilibrating with relative ease. In the case of the lipoid phosphorus, there is in man a positive gradient accounting (apparently) for its passage from mother to foetus, but in the Ungulata, where there is much more tissue between the two blood streams, the gradient is an opposition one preventing transfusion, yet it takes place. This emphasises the fact that the efficiency of the placenta as a permeable membrane does not depend upon optimal conditions for physical diffusion, but rather upon the sum total of all processes, some of which act despite apparently opposing gradients. These last are very efficient, as shown by the relative stages of development of the new-born kid, kitten and infant. Of course there are other indexes of efficient development than muscular activity, but muscular development is not able to proceed adequately without sufficient oxygen and food in the intrauterine period of tissue growth.

The experiments of Louis Flexner and his colleagues show well how permeability can be measured and compared by means of sodium chloride labelled with radioactive sodium. But this shows further, that while permeability is increased in different morphological types in rough proportion with the decrease in the number of layers between the two blood streams, there is not the same degree of freedom of diffusion achieved as in permeability between blood and tissues in adults. Further, they show that the placental layers offer varying obstruction to sodium chloride diffusion, the cytotrophoblast offering the most resistance, its disappearance yielding much greater permeability.

In addition to the permeability of the placenta to sodium chloride which Flexner has illuminated, there is the permeability to calcium and phosphate and to iron. In the cases of calcium and phosphate, the gradient is from the mother to the foetus, but what is apparently of greater importance is that if there is any shortage of available calcium or phosphate, so that the foetal blood calcium might fall below the threshold necessary for adequate calcification, then the maternal bones and teeth decalcify. This operation of the Theory of Partition of Nutrients means that wherever it happens (and it happens with iron very strikingly) the high foetal metabolism is affecting the maternal metabolism across the placenta.

Variation in Permeability at Different Ages

It is pertinent to consider here a limited number of observations on the actual passage of materials at different placental ages. Lell, Liber and Snyder (1935) found that phenosulphonephthalein could be injected into the foetal back muscles

or amniotic sac of the rabbit and detected subsequently in the maternal urine. They observed that at the 22nd day of foetal life, 30 per cent. of the back-injected dye left the foetus and 4 per cent. of the sac-injected, whereas at the 29th day only 20 per cent. of the former and 40 per cent. of the latter appeared in the maternal urine. There was at about full term, therefore, less material going from the foetus backwards across the placenta, but materials in the amniotic sac were traversing in the same direction with greater ease. These experiments had arisen out of the earlier observation of Snyder and Hoskins (1928a, b) that if endocrines, such as insulin, adrenalin, pituitrin and parathormone were injected into the foetus, no signs of their action was detectable in the mother. Lell, Liber and

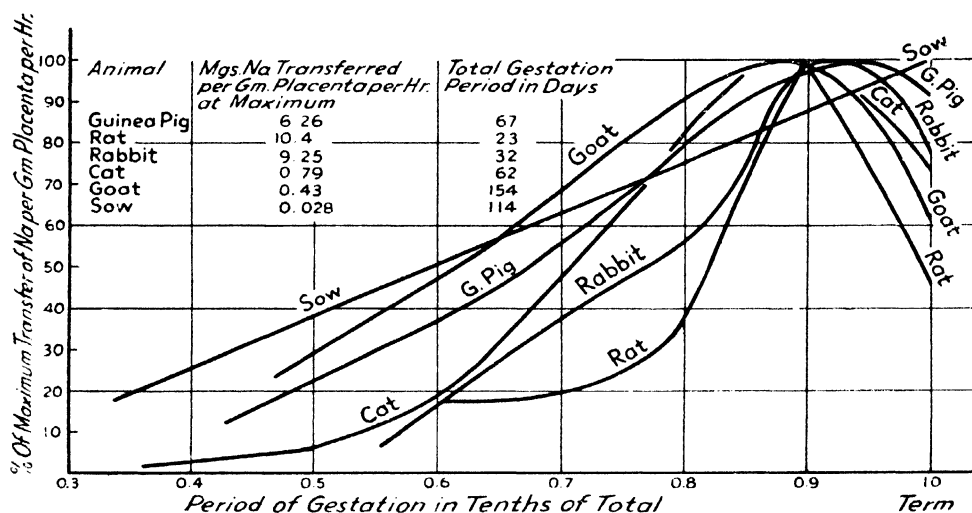


Fig. 16. 23--Graph showing transfer of sodium per gram of placenta per hour at different ages in different species expressed as a percentage of maximum transferred. (From Flexner and Gellhorn, 1942.)

Snyder used phenosulphonaphthalein because of its ease of detection, and showed it could go backwards.

In 1932, Corey showed that not only was the rat foetal blood sugar less than the maternal (though approximating to it at full term), but that if insulin was injected into the mother it freely passed into the foetus in the last third of pregnancy. Further, at this same time the placenta was freely permeable to insulin injected into the foetus, it producing a fall of maternal sugar. He found that permeability was greatest at age lengths 27–29 mm. and 39.7 mm., but that at full term the back permeability to insulin was reduced to that of the 13 mm. foetus. If these lengths be fitted to the known ages of foetal rats, in which full term is reached in 22 days $\pm$ 1 day (Huggett and Pritchard, 1942), 27–29 mm. corresponds to 19 days foetal age, 39.7 mm. to 21 days, and 13 mm. to the 15-day-old foetus. The terminal decrease in permeability on that day is possibly associated with placental separation rather than alteration in physical properties. Put briefly, therefore, Corey's work shows a progressive increase in permeability between the 15th day

and 21st days of pregnancy. On the other hand, bromine and iodine salts penetrate less easily with placental development in the dog, rabbit and guinea-pig (Birguer and Afanasiev, 1936).

The work of Flexner and Pohl and their colleagues, using radioactive sodium, shows a progressive increase in placental permeability in all species as pregnancy progresses, together with the terminal decrease found by Corey. They also showed a relative increase in different species with progressive thinning from the

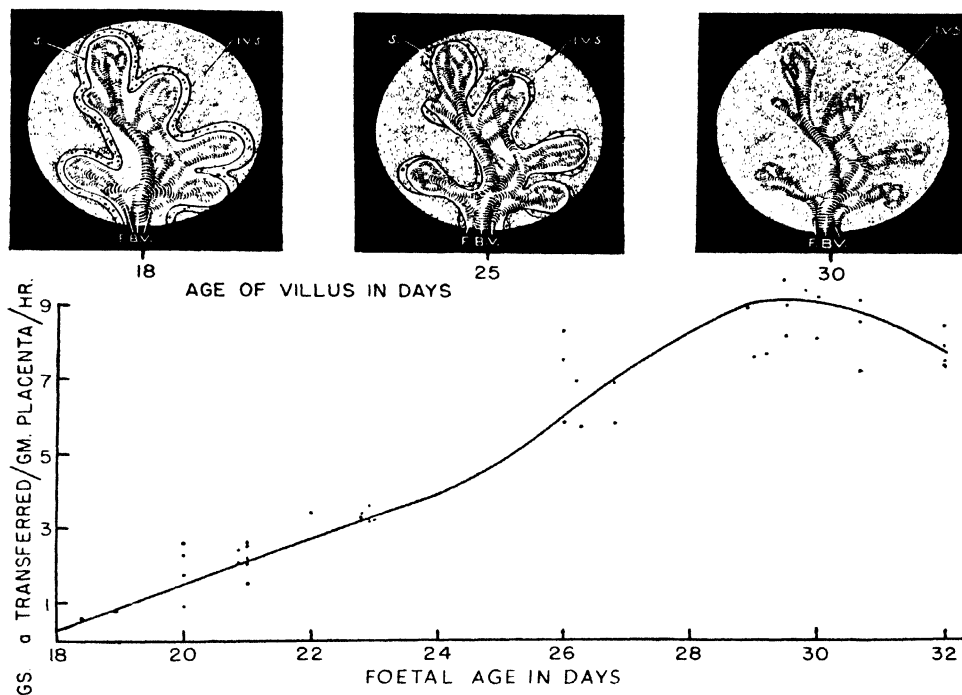


Fig. 16. 24- Graph showing the correlation between the structure of the chorionic villus of the rabbit and the permeability to sodium at different ages. (From Flexner and Gellhorn, 1942.)

epitheliochorial type to the haemoendothelial type of the guinea-pig (Flexner and Gellhorn, 1942, Fig. 16. 23, Table V). These observations agree with those of Mossman (1937).

VI. THE PLACENTA AS AN ENDOCRINE GLAND

The concept of a placenta acting as an endocrine organ arose in the first decade of this century partly out of the general interest in ductless glands, arising from the interest in the thyroid, the suprarenal and the pituitary; partly out of the endeavour to ascertain how the maternal economy adapted itself to pregnancy and to solve the question of how uterine contents could come to alter the mother's metabolism.

In 1900 post-mortem globules observed in the placenta were regarded (Natan-Larrier) as an internal secretion responsible for altering maternal metabolism. They were not visible in life. Three years later Dixon's interest in pressor extracts of organs caused him to test the placenta with positive results (Dixon and Taylor, 1908) which were not confirmed by later workers.

The ovaries can be removed in a pregnant woman without terminating pregnancy. This led Halban (1905) to put forward the view that the placenta controlled mammary gland development and that the ovarian function was transferred during pregnancy to the placenta. Starling (1905), on the other hand, attributed this function to the foetus rather than the placenta.

Numerous enzymes and hormones can be extracted from the placenta, some of which exist in quantities which are not apparently necessary, but there is not any other good reason to regard them as being specific internal secretions of the placenta. Such is acetylcholine, which is present in extreme amounts in some placentae without any obvious reason.

It appears that the placenta secretes the following hormones with useful functional effects during pregnancy—oestrogen, progesterone and gonadotrophin.

Before, however, giving the evidence for this it is worth while considering the general criteria which can be applied to an organ to determine whether or not it secretes material, and particularly whether it is not merely storing material made elsewhere. This latter is important in the case of the placenta, as the endocrine functions attributed are also activities of other proven endocrine glands, from which, therefore, distinction has to be made.

The general criterion of a ductless gland demands that its removal will cause a series of symptoms which are cured by grafting it back into the body. These deficiency symptoms may also be cured by implantation (definable as transitory grafting in which the "taking" is not permanent), by injection or ingestion of the organ or extracts of it, and finally, by the preparation from it of the pure chemicals (active principles) which on administration cure the symptoms of deficiency. Further, extracts of the organ when administered in excess will reverse the picture, and show that to be expected from hypersecretion. Clinically these experimental findings are often confirmed by the symptoms of deficiency or hypersecretion associated respectively with pathological destruction or hyperplasia. The mere presence of substances within the organ which can be extracted and which cause physiological activity elsewhere when injected is not in itself proof of endocrinal action: it may well be due to storage products or katabolites, though if treated quantitatively it may in certain cases form strong circumstantial evidence of continued production within the organ, in excess of possible storage amounts. There is, however, a danger of speculation in this since the limiting factors of storage are not clearly known. Strong evidence is obtained, however, if explants in tissue culture synthesise the hormone being investigated.

Finally, there is the demonstration of the active principle in the blood stream as well as in the organ and establishment of its chemical constitution together with its isolation and synthesis and the preparation of other substances of similar structure and biological action.

When we come to apply these criteria to the placenta we are faced with the fact that it is itself a transitory organ. Certain functions it exercises (e.g. transport of oxygen and food to the foetus) are essential for foetal life and also for continua-

tion of pregnancy, that if these are alone terminated quite a number of symptoms arise for which there is no need to assume endocrine action in the placenta. They cannot, of course, be cured by grafting or by extracts of placenta.

Evidence for Gonadotrophin Secretion

Alkaline extracts of the anterior pituitary cause luteinisation of the ovary in immature rats (Evans, 1924) without causing folliculisation. Implants of anterior lobe tissue (from non-pregnant, but not from pregnant, women), however, cause follicle maturation, ovulation and oestrin secretion (Zondek and Aschheim, 1927). Similarly, implants of placental tissue in rabbits produce gonadotrophic responses (Hirose, 1919). In pregnancy, human urine contains material which, when injected into immature mice, causes folliculisation with haemorrhage and luteinisation (Zondek and Aschheim, 1928). In rabbits, injection of human pregnancy urine causes ovulation and pronounced luteinisation (Friedman, 1929); normally, rabbits never ovulate unless they have previously copulated, therefore the urine must have contained gonadotrophin. These observations on gonadotrophin form the basis of the Aschheim-Zondek and the Friedman tests for pregnancy.

Gonadotrophin from the anterior pituitary gland (AP) has been fractionated (Fevold, Hisaw and Leonard, 1931) into two substances, the luteinising and the follicle-stimulating hormones (LH and FSH). That in pregnancy urine is denoted as PU and differs apparently from AP in numerous respects. PU is gonadotrophic like AP, but is less effective in the hypophysectomised animal, whereas AP offers complete replacement. The problem is discussed by Newton (1938). PU appears to be ineffective in the immature animal compared with AP. The difference between the two extracts is the less the older the animal, that is, the longer the gonads have been under the influence of the pituitary before the hypophysectomy. Another difference (Fevold and Hisaw, 1934) is that there is a synergistic effect between subminimal doses of PU and FSH or between placental extract and FSH, such that the two together have an effect greater than the summation of their individual doses. This synergistic effect is exactly the equivalent of that between FSH and LH; PU is therefore thought to resemble LH rather than FSH. This is supported by the fact that LH and PU have no synergistic action. The blood serum in pregnancy also appears to contain gonadotrophin which behaves similarly to PU rather than AP (Boycott and Rowlands, 1938). (See Chapter 11.)

The gonadotrophin of pregnancy urine is therefore different in action from that of the pituitary body. It may also be different in origin. It is tested for by its action in causing folliculisation or luteinisation or both, particularly in hypophysectomised animals. The evidence of its origin from the chorion can now be set forth.

Gonadotrophin is detectable in the urine in all conditions in which there are chorionic tissues present, disappearing with their complete removal as with normal parturition and complete abortion (von Arvray, 1934). Ware and Main (1934) and Frank (1929) have described cases of abdominal pregnancy in which the foetus was removed, but the placenta, being attached to the abdominal viscera was left. Gonadotrophin was excreted in the urine for some considerable time after the foetal removal.

Chorionepitheliomata, without attendant foetuses, can be found not only in "pregnant" women but also occasionally in non-pregnant individuals, notably children and men. Heidrich, Fels and Mathias (1930) described a case in man where the primary growth having been removed, the secondaries being present, gonadotrophin persisted in the urine. Similarly, Siegmund (1932) described the case of a small girl who excreted it in her urine after her primary growth had been removed, the secondaries being still present. Tenney and Parker (1939) estimated the excretion of gonadotrophin in the urine in a series of cases of chorionepitheliomata and determined it as proportioned approximately to the number of cells in the placenta or "mole." There was less where cysts predominated and cells were relatively scanty. This suggests again that the cytotrophoblast is its source.

There is evidence that the placenta forms different types of gonadotrophin at different ages; in early pregnancy it forms mainly a luteinising type and in late pregnancy mainly follicle stimulating (Philipp, 1930a). A point of great importance is Philipp's (1930b) discovery that in pregnancy the anterior pituitary loses its gonadotrophic (both LH and FSH) action. This has been confirmed several times, notably by Saxton and Loeb (1937), and appears substantiated, though its significance is unknown. In 1936, Philipp and Huber showed that gonadotrophin was demonstrable in the trophoblast in cases of abortion in seven out of twelve cases, but in the decidua in only three out of seven of these cases. When found in the decidua it was also in the trophoblastic part of the placenta. They inferred it is made in the trophoblast and spreads to the decidua. This synthesis of the hormone by the chorion is supported by an interesting experiment of Kido (1937). He determined the minimal amounts of human chorion which, if implanted in a muscle or if extracted and injected, would give positive responses. He then implanted less in the eye of a rabbit than these amounts. The heterologous graft "took." The rabbit secreted a urine containing gonadotrophin—as shown by its yielding a Friedman test in a second rabbit. Kido inferred synthesis of gonadotrophin by the implant. It might have been stimulation of the first rabbit's pituitary by the implant. It has not been repeated on a hypophysectomised rabbit, but is nevertheless suggestive.

In tissue culture chorionic villi develop the cytotrophoblast, but not the syncytiotrophoblast. Gonadotrophin is recoverable in active culture even after months, but not the steroid hormones (Jones, Gey and Gey, 1943; Gey, Jones and Hellman, 1938). This again indicates the cytotrophoblast as the source of gonadotrophin.

Not only does gonadotrophin appear in human urine (where it is mainly luteinising), but also in the pregnant mare (where it is follicle-stimulating) in its blood serum and in the decidua and chorion (Cole and Hart, 1930; Catchpole and Lyons, 1934). It appears in the blood from the time of contact of the chorion and decidua (Glud, Pedersen-Bjergaard and Portman, 1933; Catchpole and Lyons, 1934). In human urine the peak period of PU gonadotrophin is between the 40th and 50th day of pregnancy. This is the date of invasion of the ectodermal chorion by the mesodermal allantois (Browne and Venning, 1936; Evans, Kohls and Wonder, 1937). In the mare the similar date is the 60th day. Newton (1938) endeavours to correlate the production of gonadotrophin with this fusion of allantoic circulation and trophoblast to form a chorion. This is of interest

since Ewart pointed out (1915) that this date was critical for the production of spontaneous abortion, and it also appears that if the rat foetus be killed before the fusion of the allantoic circulation with the trophoblast, absorption will occur, but if this is established, the formed placenta will persist to full term (Huggett and Pritchard, 1945a, b and c). When, in an established human pregnancy, fibrosis of the tissues between the allantoic circulation and the maternal circulation occurs (Klein, 1933), then the Ascheim-Zondek reaction goes negative. Abortion follows.

Evidence for Oestrogenic Hormones

There are two available criteria of oestrogen secretion by the placenta, the demonstration of an oestrus producing hormone by the general methods available for identifying an organ as a ductless gland and, secondly, the isolation of a secretion in the blood or urine of the chemical configuration of oestrone. This last is not available for the gonadotrophins which have not been chemically analysed.

During pregnancy the oestrogen content of the urine falls and then rises at the end (Probstner, 1931) to normal values. If oöphorectomy be performed during pregnancy the oestrogen content of the urine falls (Allan and Dodds, 1935) in the woman, but pregnancy is not interrupted. In the mare, likewise, oöphorectomy during pregnancy also does not interrupt pregnancy and the excretion of oestrogens goes on in amounts far exceeding for the whole of pregnancy the quantity which might have been stored in the placenta, suggesting synthesis by it (Hart and Cole, 1934). Cohen, Marrian and Watson, in 1935, showed that the oestrogens in the urine are in human pregnancy in four forms, namely, oestriol, free and combined, and oestrin, free and combined. The free oestrogens increase in the urine about 12-15 days before labour and then fall to have a secondary rise at labour, while the combined oestrogens behave in the reverse manner. They exceed the free oestrogens in the urine, but decrease at about the 12th day and recover, to decrease again at about labour. This explains why there is much total oestrogen in the urine in pregnancy, yet the injection of free oestrogens terminate pregnancy. In cases of chorionepitheliomata, oestrogens are sometimes present in the urine. In Siegmund's case (1932) already referred to there were none, but de Snoo (1928) does report a case in which Laqueur demonstrated 300-1,000 MU of oestrogens after both ovaries and the uterus had been removed and metastases had appeared. Philipp (1929), in an endeavour to demonstrate the production of oestrogen by implantation experiments in castrated mice, obtained mixed results. These were negative when he implanted ovaries from a sow which was in advanced pregnancy—suggesting absence of oestrogen secretion at that stage. They were positive with implantation of the foetal portion of the placenta of the sow, but negative with implantation of the rabbit's placenta.

Aschner (1913) first showed placental extracts caused uterine growth, and Doisy, Ralls, Allen and Johnson (1924) first showed such extracts give the characteristic oestrus smear in immature mice. Oestrogens have been demonstrated (vaginal smear test) in all animals except pig, dog, cat and rat, though, as has been pointed out, Philipp (1929) got positive results for the foetal placenta of the pig by the implantation test.

The quantity of oestrogen in the placenta is of the order of 500-5,000 MU per placenta with mean contents of 1,000-2,000 MU (*see* Newton, 1938).

Clauberg (1936) thinks it is manufactured by the placenta since it is in higher concentration than in the ovary ; however, it is also in still higher concentration in the kidney (Catchpole and Cole) ; presumably, this is an argument for storage rather than secretion.

The identification of the pure oestrogens has led to the isolation from human placenta of oestriol (Butenandt and Browne, 1933), oestrone (Westerfield, *et al.*, 1938) and in the urine of non-pregnant women of oestradiol (Marker, *et al.*, 1938). Marrian and his colleagues have shown that during pregnancy the urine contains relatively little free oestriol and oestrone, but a relatively high quantity of the congregated forms (glycuronides) (Cohen, Marrian and Watson, 1935 ; Cohen and Marrian, 1935).

The decrease in urinary oestrogens (free and combined) after delivery, the continuance during pregnancy after ovariectomy and the relatively high concentration in the placenta suggests that they are probably produced in the placenta, though storage is not excluded, but is unlikely in the absence of the major known source (the ovary).

Evidence for Luteal Activity

Removal of all the foetuses in the rabbit (Giacomini, 1893) did not stop the placentae growing. But if the rabbit's ovaries be removed the pregnancy terminates (Fraenkel, 1903). This is not the case, however, after the 13th day in the rat (Marshall and Jolly, 1906) ; further, in the guinea-pig the corpora lutea are only needed for the first six days of pregnancy (Loeb and Hesselberg, 1917). In rabbits, however, they are needed throughout pregnancy (Corner, 1908). It would appear that the ovaries or corpora lutea are needed throughout pregnancy in the cow, sheep, rabbit, squirrel, mouse and opossum, but ovariectomy can be performed in the latter half of pregnancy in man, the Rhesus monkey, the mare, guinea-pig, the cat and the rat without terminating the pregnancy.

Hammond (1917) found in the rabbit that removal of the foetuses permits persistence of the placentae, but all signs of pregnancy disappeared. On the other hand, in the mouse, if this operation be performed, both the placentae and the signs of pregnancy persist (Newton, 1935). Similarly, in the rat, when all embryos are removed between the 9th and 13th days, signs of pregnancy last till full term, when placental delivery occurs (Selye, Collip and Thomson, 1935).

It would appear, therefore, that the signs of pregnancy, and the restraint of uterine activity associated with a pregnant uterus in some animals, depend upon the corpora lutea and in other animals upon some other agent than the ovaries. Haterius (1936), using the rat, removed all foetuses bar one, but left all the placentae behind. He then removed both ovaries and found the remaining foetus remained intact to full term, but if he initially removed all foetuses and also all placentae except one foetus and its placenta, then an oöphorectomy, abortion occurred. He inferred that the multiple placentae secreted sufficient progesterone to compensate for the loss of the ovarian source. Kirsch (1938) produced evidence in favour of the placenta prolonging pregnancy signs. Using the rat he removed all foetuses after the 14th day ; all the placentae went to full term at the 21st day. If he added to this ovariectomy, and reduction of placentae to six, one-third of the placentae held on to full term, one or more aborting. When he completely removed all foetuses and their placentae, but replaced them by paraffin wax pellets

simulating the local distensions of the sacs, he found all pellets were aborted, but if he put the pellets in one horn only and left the embryos in the second, then the pellets and embryos were all delivered together at full term. He concluded that the placenta controls the length of gestation and some functional placenta must be present for this to occur.

These experiments of Kirsch were followed by Haterius and Kempner's repetition (1939) of the early work of Haterius. They found that if, when both ovaries were removed, one foetus and one placenta being left, paraffin pellets were inserted, then the pregnancy persisted to full term. In other words, pellets appeared to replace the placenta or rather the placenta appeared to act mechanically rather than hormonally.

In 1943 Zeiner repeated Haterius' work, but he performed the ovariectomy with care and without trauma in two stages at the 13th and the 15th days. He found that the foetuses and placentae persisted to full term irrespective of the number of placentae in excess. In 1945 Huggett and Pritchard (1945a), using the rat, killed the foetuses. They found if killing was performed after the mesoderm and allantoic vessels had grown into the ectodermal trophoblast, then the embryo went to full term, but if before this date (before the 11th day), then the trophoblast grew till the 16th day when it was destroyed by maternal haemorrhages into the cells of the trophoblast where spaces were left by glycogen disappearance (which normally is maximal at the 16th day). It would appear, therefore, that in the absence of the ovaries the rat placenta is delicate but will, if untraumatised, go to full term. This ability to survive is dependent upon its integrity and its own allantoic blood supply.

Newton has pursued the matter further. Besides showing that the placenta plays an important part in inhibiting oestrus and determining the date of parturition (1935), in the retention of water in pregnancy (Brooksbey and Newton, 1938), in the development of the mammary gland and the lengthening of the interpubic ligament during pregnancy (Newton and Lits, 1938), in the maintenance of body weight (in conjunction with the ovaries) and in mammary development (independent of the ovaries) (Newton and Beck, 1939), he further showed with Richardson (1940) that it is even responsible for milk secretion in the last week of pregnancy and also for persistence and growth of the corpora lutea during pregnancy, elimination of the placentae causing regression of the corpora lutea (Deanesly and Newton, 1940).

In conjunction with van Wagenen (1943) he has shown these mouse results apply to the Rhesus monkey. Removal of the foetuses from eleven monkeys, leaving the placentae, gave many signs of pregnancy, notably the normal growth curve, sex skin colour, generalised oedema, physical inactivity, onset of labour at normal term, and the increased excretion of androgens and oestrogens of normal pregnancy with readjustment after pregnancy.

Active extracts with a progesterone-like activity have been obtained from the human placentae (Ehrhardt, 1934; Ehrhardt and Fischer-Wasels, 1936). The extracts need to have the oestronic substance removed before testing (Adler, de Fremery and Tausk, 1934; McGinty, McCullough and Walter, 1936), who also obtained a progestin reaction from the cow's placenta, and by Smith and Kennard (1937), who showed placentae from toxæmic cases had the same content of progestin and oestrogen.

Wislocki and Bennett (1943) have demonstrated that the syncytiotrophoblast gives histochemical reactions for ketosteroids, namely phenyl-hydrazone formation which disappears after acetone extraction. This they associate with progesterone production in the syncytium.

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CHAPTER 17

FOETAL RESPIRATION AND CIRCULATION

By JOSEPH BARCROFT

The morphology of the placenta has formed the subject of a previous chapter of this volume. We now turn to the foetus. As development of the foetus may be supposed to be in some way dictated by the placenta, a word may be said about the connection between the two, the moral being that the connection is quite obscure. Among those mammals which are most highly developed at birth, the horse, the cow and the sheep are those in which there has been no, or but little, reduction in the layers which constitute the placental barrier, yet the guinea-pig, also well developed at birth, has a placenta with but one layer. If there is any connection between the reduction of the layers and the development of the foetus, it may be that mammals which develop rapidly in the first three weeks from insemination show a considerable reduction in the layers constituting the placental barrier. Yet even this is not established as involving cause and effect.

In the study of pre-natal life it is interesting to keep an eye on the possible factors which make for efficiency in the forms of placenta in which the epithelial layers are retained, for to take the extreme case of the placenta consisting of no more than a barrier such as that presented by the pulmonary epithelium, it could do little more than act as a mechanical filter ; but let us turn to the foetus itself.

I. DIMENSIONAL GROWTH OF THE FOETUS

The increase in length (crown-rump length) is much more regular than the increase in weight, and in the case of the sheep, for instance, over the last two-thirds of gestation the crown rump length increases in an almost linear proportion to the foetal age. Not only is the linear relation, as expressed (Fig. 17. 1) by the graph, striking, but so also is the small scatter of the points relevant to any particular age. This scatter increases somewhat just before birth, but even then it is not large. It is, however, a hint of the much larger scatter observable at the end of gestation when many other properties of the foetus are correlated with its age. There seems to be very little difference as between the age-length curve of singletons and twins respectively in sheep.

In general, the foetus puts on more weight each day than on the day which precedes it, but the rate of growth, i.e. the increment in weight in a given time interval expressed as a percentage of the mean weight during that interval tends to decline as gestation proceeds.

Much has been written in the endeavour to express the daily increments of weight and the rate of growth by mathematical formulae. The difficulties facing such projects are very great—at all events, in the case of mammalian foetuses.

Two such difficulties may be mentioned. The foetus is composed of organs which develop at different times (Carlyle, 1945b). There can be no assumption, therefore, that the time-weight curve of any particular foetus should be a smooth curve at all, or susceptible of mathematical treatment as representing any specific property in the growth of the individual; that is the first point. The second refers to the growth of the individuals, the data concerning which are averaged to arrive at the sort of time-weight curve used as the basis for statistical analysis. Fig. 17. 2, which refers to the sheep, indicates that the lightest foetus at term is scarcely heavier than the lightest foetus at 120 days, whilst the heaviest foetus at term is

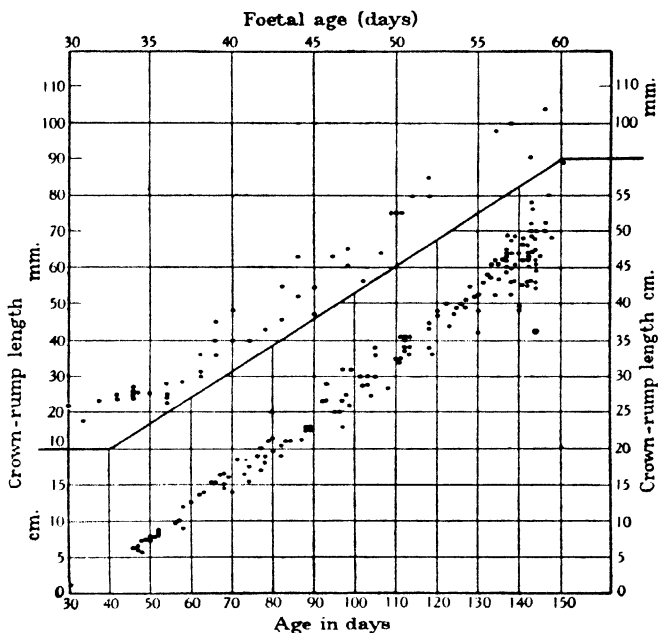


Fig. 17. 1—Relation of crown-rump length to foetal age (sheep).
(From Barcroft, 1946.)

perhaps twice the weight of the heaviest foetus at 120 days. This suggests that some foetuses falter in their growth at about 125 days, whilst the growth of others goes on more or less unabated. In some mammalian forms, such as the pig, this faltering is much more pronounced than in the sheep. If the uterus in the sow be opened, foetuses of quite different sizes will be found, many of which have come to a standstill so far as growth is concerned. These would be reabsorbed and at a later date would have disappeared. Thus the time-weight curve obtained from the average of foetuses found in the uterus really has little relevance to the growth of any particular foetus.

In spite of the improbability of the time-weight curve being the mathematical expression of anything particularly revealing, the fact stands out that the great increase in the weight of the foetus is in the last quarter of pregnancy. The practical importance of which is the probability that during that time malnutrition

of the mother will have the most prejudicial effect upon the foetus. If, as may be supposed, the normal singlet foetus is exploiting to the full the powers of its mother to nourish it, it may also be supposed that were there twins they would be undernourished in some degree. Actually, in the case of sheep, twins are on the whole slightly lighter than singlets at birth, though it is only within the last few weeks of foetal life that the difference in weight becomes appreciable. There seems to be very little difference in length as between lambs at birth, be they

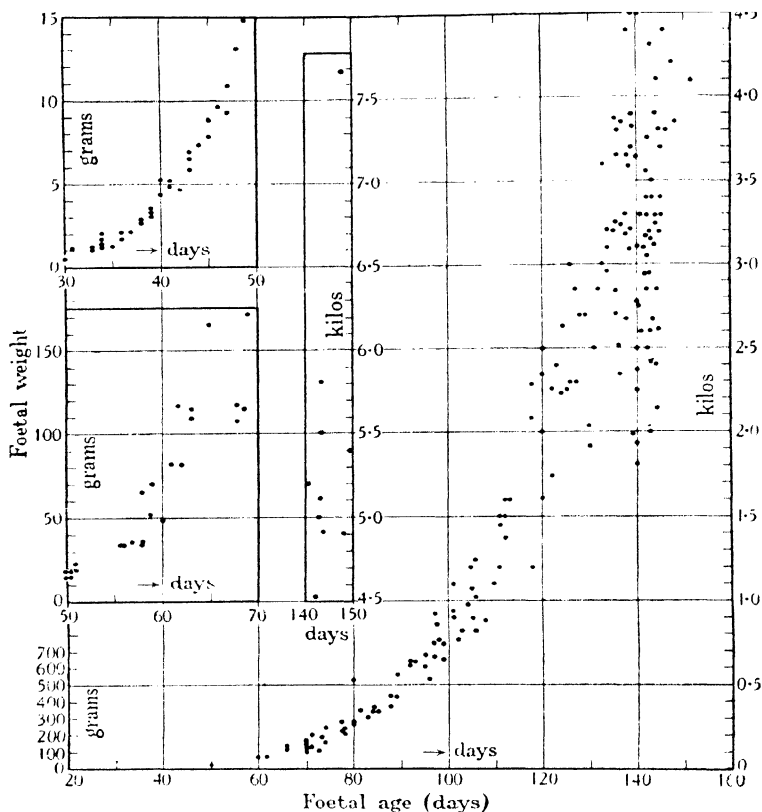


Fig. 17. 2—Relation of weight to foetal age (sheep).
(From Barcroft, 1946.)

singlets or twins. As the crown-rump length is almost entirely a question of the growth of bone, it follows that bone, so far as its growth is concerned, is less sensitive to undernourishment than many other tissues.

The above considerations raise the question: Is it possible experimentally to produce foetuses under the normal weight by restricting the diet of the mother? So far as sheep are concerned, the answer is certainly "Yes." With regard to man, the answer seems to be uncertain. The general impression with regard to women is that the foetus will exploit its mother "one hundred per cent.," and will maintain its rate of growth at whatever cost to its parent.

To take first the easier case of the sheep, in which strict experiment is possible. The recent experiments of Wallace (1946) are very convincing. The following is an example of the results obtained :—

TABLE I

Weight of ewe (kilos).				Weight of foetuses.		
At service.	91 days.	144 days.		144 days.		
Diet,) High Level)	(61·5 (61·8	72·2 78·8	91·4 95·8	Singlet Twins	6·07 5·00	5·36
Diet,) Low Level)	(61·4 (62·8	58·2 56·0	51·3 50·4	Twins Twins	2·70 2·77	2·62 2·36

Further, Wallace showed that it was almost immaterial whether the ewe was on a high or low level of diet except during the last third of gestation. These experiments were conducted with great care in the selection of strictly comparable stock throughout. It is certain that in sheep insufficient nourishment during the last third of pregnancy will show itself not merely in reducing the weight of the mother, but in stunting the lamb.

It is difficult to obtain any convincing data about man which prove either that the weight of the offspring at any given foetal age is unaffected by the reduction in the diet of the mother or the reverse. Certain it is that the babies of ill-fed mothers are on the whole somewhat lighter at birth than the babies of well-fed mothers, but the former include a considerably greater number of prematures, which, of course, are lighter than they would be had pregnancy run its full course. The ill-fed mother has also given birth to a greater number of still-born children. From the sociological, as opposed to the scientific, point of view, it is perhaps not very material whether the average weight of the offspring of undernourished babies is reduced by prematurity, by still birth, or by a uniform reduction in the weight of the foetus at a given age. The moral is that in one way or other the foetus is on the whole prejudiced if the mother during the later portion of pregnancy is underfed (Dougal Baird, 1945).

Possibly, as the result of researches undertaken during periods of gross starvation during and after the war, we shall get definitely to know whether inanition of the mother will produce stunting of the child during intra-uterine life. One such research has been carried out by Dr. Clement A. Smith (1946) of the Harvard University Medical School. Only a quite preliminary report has so far been published, but the main conclusions are as follows :

(1) The babies born of mothers underfed at the end of pregnancy were smaller than normal. This applied whether the prematures were included in this count or not, but was true even if only 40-week babies were taken into account.

(2) In babies born as little as three months after the relief of famine, the rise of birth weights becomes less marked.

II. THE WATER CONTENT OF FOETAL TISSUES

It has been known for long that the water content of many foetal tissues decreases with foetal age (*see* Needham 1931). More recently, researches have been carried out independently by Davidson and Waymouth (1944) and Carlyle (1945a). The former are on the chick and the 9-10-week-old sheep foetus. The latter on sheep foetuses at various ages. So far as these researches overlap, there is good agreement between them. The following table is given by Carlyle (1945a).

TABLE II

SHOWING THE DRY WEIGHT OF TISSUES OF THE SHEEP FOETUS, AT DIFFERENT AGES, AND OF ONE LAMB, EXPRESSED AS A PERCENTAGE OF THE FRESH WEIGHT

	Age of foetus (in days).								Lamb, 6 days.	Adult.
	72	78	103	112	120-2	126	135-6	142-4		
Skin	9.8	10.6	12.8	—	16.1	18.5	24.2	21.9	22.3	29.6
Bone	27.1	33.9	49.0	—	42.0	34.0	46.6	45.4	56.0	67.0
Cartilage	—	—	21.4	—	29.0	—	20.6	22.5	22.0	35.0
Muscle	9.6	10.1	11.4	—	17.0	18.5	17.8	19.2	20.7	26.6
Liver	17.6	19.4	19.3	—	19.2	18.9	21.0	26.1	23.0	30.0
Gut	12.2	12.2	—	14.8	14.5	15.1	13.2	14.1	17.1	22.3
Lung	9.7	11.6	11.1	—	11.1	9.3	11.4	14.5	21.5	21.2
Brain	10.6	9.5	—	10.4	—	—	13.2	15.6	15.2	24.3
Heart	12.5	—	—	15.3	—	—	—	12.2	19.0	18.3
Kidney	12.5	11.9	—	8.1	—	—	—	11.4	18.9	21.9
Blood	—	—	—	15.8	16.6	18.9	16.6	17.1	21.1	19.7

Obviously, different tissues show great disparity in the percentage of dry matter which they contain at different ages. Muscle, for instance, contains over 90 per cent. of water at 72 days and about 81 per cent. at term. Heart, on the other hand, has substantially the same water content at 72 days as it has just before birth and the kidney has slightly less solid matter at birth than at 72 days. In some cases, such as the skeleton, there is an obvious reason for the change, such as the laying down of calcium phosphate.

III. THE GREAT VESSELS IN THE CHEST

The foetal heart differs from that of the adult principally in that the left and right sides are of approximately the same size, and that the left and right auricles are reputed to be in connection with one another by means of the foramen ovale. It is not the object of this chapter to deal with the strictly anatomical and histological matters: nevertheless, it is difficult completely to dissociate the consideration of function from that of structure, for which reason something must be said about the structure of the auricles and the vessels leading to them.

Recently the whole subject of the avenues along which blood reaches the heart, and the course which the blood takes on arrival, have been reviewed by Barclay, Franklin, Prichard, Amoroso, Barron and Barcroft, working either together or separately. The subject may be treated the more lightly here in view of the issue of Barclay, Franklin and Prichard's (1944) book, *The Foetal Circulation*.

The general results of this review have been to show :

On the anatomical side the superior vena cava is so disposed that the whole of the blood which traverses it may be expected to find its way into the right auricle, whilst the inferior vena cava is disposed so that that is not the case. Facing



Fig. 17. 3 — Sheep foetus (100-147 days). A feeble pulsation of the left heart has momentarily cleared the ascending aorta and partially cleared the aortic arch and brachiocephalic artery, thus leaving a well-defined picture of the ductus arteriosus uniting the pulmonary trunk and the descending aorta. (From Barclay, Franklin and Prichard, 1944.)

the orifice of the inferior vena cava in the heart wall, there is a pillar of tissue projecting from the wall of the intra-auricular septum, the *cresta dividens*, in which the stream of blood breaks and divides in two currents. One portion of the stream may be expected to pass directly into the left auricle, through what is functionally a small funnel and which, anatomically speaking, consists jointly of the foramen ovale and the so-called valve of the same. The other portion passes towards the right auricle, is shortly joined by the stream arriving from the superior vena cava, and these streams jointly form the affluent to the right side of the heart.

Into the left side, as well as the current mentioned above, there flows the blood returning from the pulmonary circulation.

The foregoing interpretation of the anatomical facts agrees with what the above authors have observed by method of X-ray cinematography. If the neck

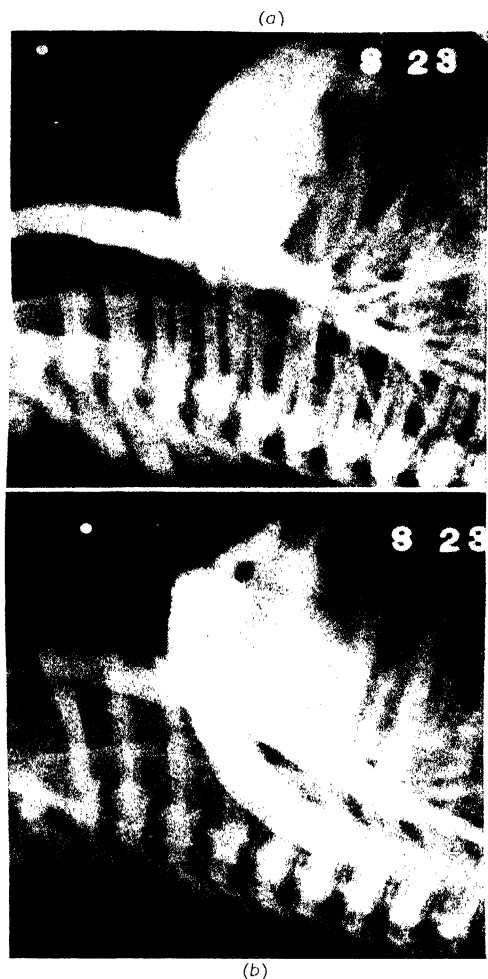


Fig. 17. 4—(a) Anterior caval injection, $6\frac{1}{2}$ mins. after delivery. Nose bag on, cord undivided. The contrast medium is passing freely through the ductus arteriosus to the descending aorta, as well as to the pulmonary arteries. (b) Another anterior caval injection $4\frac{1}{2}$ mins. later. The nose bag has been removed and the cord divided 4 mins. before this injection. The contrast medium is now passing to the pulmonary arteries only and no shadow is seen in the descending aorta, i.e. the ductus arteriosus is functionally closed. (From Barclay, Franklin and Prichard, 1944.)

of a sheep's foetus be exposed, and if a radio-opaque material be injected into the superior vena cava, the opaque material passes in its entirety to the right side of the heart and thence from the right ventricle to the pulmonary trunk: from which, at the bifurcation of that trunk, it passes on the one hand along the ductus arteriosus to the dorsal aorta, and on the other to the pulmonary arteries (Figs. 17. 3 and 4).

Radio-opaque material injected into the umbilical vein takes a different course through the heart. The major part passes into the left auricle, then to the left ventricle and the aortic arch, so feeding the coronary vessels and those to the head, neck and arms; in addition, some of the material passes on down the dorsal aorta. The major portion of the stream down the dorsal aorta is, however, that which arrives along the ductus arteriosus (Fig. 17. 4 b).

Thus, for the present, ends a long historic controversy which need not be discussed here. Opinion has oscillated from one view—that all the blood which arrives at the heart along the inferior vena cava goes to the left auricle and ventricle, and all arriving via the superior vena cava goes straight through the right auricle and thence to the right ventricle: these streams being supposed to cross in some mysterious way in the right auricle—to the opposite view, namely, that the venous blood from all sources mixed completely in the right auricle. It need only be said that the view which, on the latest information, seems to be correct, was essentially that put forward by the Russian observer Wolff as long ago as 1778.

It only remains to add that, contrary to the beliefs current till recently, both the ductus arteriosus and the ductus venosus close within a few minutes of birth. Each is furnished with a sphincter in the form of a muscle; that surrounding the ductus arteriosus is of a special character and consists of interwoven bands of

fibres disposed as right-handed and left-handed spirals ; these, when they contract, not only constrict the lumen but kink it, incidentally shortening the ductus. This closure is at first a purely physiological affair, but as time goes on the sphincter cicatrises.

The great veins.

A little more detail must be given about the great veins leading to the heart. They, also, have been studied by cine-radiological technique, this having been done by Barclay, Franklin and Prichard.

In general, the venous supply of the heart consists of three main vessels : the anterior vena cava, the azygos and the posterior caval channel. The background of the posterior caval channel consists of (1) the inferior vena cava and (2) an arcade into which opens at one end the portal vein and at the other the umbilical vein. Connecting this arcade with the posterior caval channel are a number of vessels, some of which traverse the liver, whilst one—the ductus venosus—leads directly from the arcade to the posterior caval channel, by-passing the liver.

IV. THE PULSE

The development of the pulse-wave.

Pickering (1893) in his classical paper on the development of the heart-beat says, as the result of studies made during the interval between the first appearance of the beat and the time at which the heart comes under nervous influence : “ The normal contraction wave passes from the entrance of the veins into the auricle and thence to the ventricle. If a small piece of silk fibre be placed round the auroventricular junction, partial blocking of the auriculo-ventricular sequence can be obtained, so that for every two or three auricular beats, only one beat of the ventricles results.”

The above quotation no doubt represents what Pickering said, but if it is taken to mean that the cardiac impulse appears in the sinus while as yet none has been developed in the chamber lower down, such gloss would be erroneous. Indeed, the first appearance of muscular activity in the heart can scarcely be termed a cardiac impulse at all.

The earliest manifestations of contraction in the myocardium have been observed by Johnstone (1925) in the chick, and in the rat by Goss (1938). Johnstone says, “ Continuing the work of Dr. Sabin on the development of the heart-beat in chick embryos, it was found that the first pulsations appear at the ten-somite stage, and that the beat at this time is confined entirely to the primitive ventricle. Furthermore, it was observed that the first spot in the primitive ventricle to beat was on the right border, and occurred typically about three-eighths of the distance from the venous to the arterial end of the ventricle. The beating area then spread along the right border of the ventricle until gradually the entire border commenced to contract. When about three-fourths of this border had begun to beat, the contractile area had reached the left border of the ventricle. The area of contraction first involved the left border at a point about a third of the distance from the venous to the arterial end of the ventricle. It then spread along the lesser curvature in a manner similar to its progression along the greater curvature, until

gradually the entire ventricle began to contract rhythmically. During the period when the heart-beat was developing, and for a long time after the primitive ventricle was pulsating rhythmically, there was no movement of the primitive sinus venosus . . . as the primitive ventricle gave rise to the primitive atrium, permanent ventricle and bulbus arteriosus . . . the atrium became the dominant rhythmical area of the heart. It was not possible to modify the beating of these chambers at this stage of development by separating them from the primitive sinus venosus with a ligature. By the fourth day of development, however, conditions were found to be changed; the isolation of the rest of the heart from the sinus venosus by ligature produced heart block."

The above observations were repeated and expanded by Patten and Kramer (1933), who observed that the order in which contractivity develops is that in which the chambers are actually formed. They also date the commencement of the blood-flow " . . . after a period during which the peristaltoid contractions initiated in the atrium gradually increase in power and rate, and the blood is set in motion. When the circulation thus commences the sinus is still unformed."

All this work has been repeated on the developing heart of the rat by Goss (1938) in which the first traces of a beat are recognisable at approximately 9½ days, though there may be a difference of 4-6 hours as between the heart of one embryo and another in the same litter. The first contractions have a rhythm of 37-42 per minute and were confined to the ventricle. The right myocardial tube commences contraction two hours before the left and with a slower rhythm; when the tubes join, the left tube is the pace-maker. This ventricular wave travels in the A-V direction. The rate gradually quickens and there is no circulation of blood.

The pulse-rate in foetal life.

The alterations in the rate of the pulse with the progress of foetal life have been studied in the chick. The most reliable results are those of Bogue (1933), which were obtained without opening the egg, and the temperatures of which were carefully controlled—a very important matter.

Bogue's results are given in Table III.

TABLE III

Duration of incuba- tion in days	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18
Average pulse- rate	149	173	197	209	217	223	228	231	234	236	237	238	239	239	240	240	238

Very similar results were obtained previously by Cohn and Wile (1925), whose counts, however, were by inspection and involved the opening of the egg. Bogue's were electro-cardiogram tracings taken from leads inserted through the shell, the currents being amplified. The results in both cases show a rapid rise within the first week of incubation, after which the rise in pulse rate becomes slow, and during the last ten days the pulse-rate is practically constant.

The literature previous to Bogue's work will be found in his paper, as will a number of isolated observations on man, the ox and the dog.

The only mammal in which the pulse-rate has been investigated experimentally through the course of pregnancy is the sheep. In the sheep, Barcroft and Barron (1945), observations have been made over the last two-thirds of pregnancy. The curve is of a different shape from that of the chick, reaching a maximum of about 200 at about the 100th of the 147 days of gestation, then falling markedly to a figure of about 130 at the end of gestation.

The cause of the final fall in pulse-rate seems to be the appearance of vagus control, which occurs at about the 100th day and becomes increasingly powerful as time goes on. With the vagi cut, the general trend of the curve in the sheep would be much like that found by Bogue, Cohn and others in the chick.

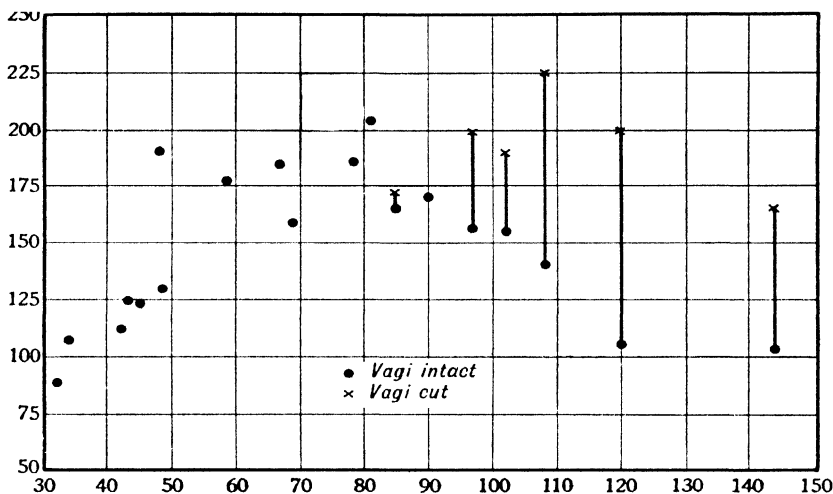


Fig. 17. 5—Pulse rate considered in relation to foetal age (sheep).

(From Barcroft and Barron, 1945.)

The finding that there is considerable vagus control in the sheep seems to be in conflict with the statements usually made: for instance, to quote Bogue, "Many observers have shown that the vagus exercises very little control in new born animals." The difference may not be a real one; the mammalian forms to which Bogue's statement refers have been such (puppies, kittens, etc.) as are born in a much less mature condition than are sheep. It is certainly true (Bauer, 1938) that vagus control in the rabbit does not appear till after birth.

Intra-uterine vagus control in the sheep (*see* Fig. 17. 5) is dependent on the satisfactory condition of the nervous system, and in order to observe it to the best advantage the foetus should not be delivered, nor should the uterus be exposed or moved from its normal position. Whilst the fact of vagus control in forms born in a high state of development seems certain, the cause is not so clear. It could be ascribed either to central or peripheral influences.

Towards the end of gestation, as will be seen later, the blood in the brain becomes progressively more asphyxial in character, and asphyxia acts as a stimulus

to vagus control of the heart ; whether the degree of asphyxia to which the brain is subjected during foetal life is sufficient to impose vagus control on the heart is another question.

It is certain, too, that at any time after about the 80th day the heart may be slowed reflexly by stimulation of the central end of the divided vagus. Thus, both the central connections and the peripheral effector mechanism are intact by that date. The receptor mechanism appears to be judged as histological—but here again, whether aortic-depressor and carotid sinus mechanisms, even if perfected, are used is another matter. According to Bauer, the corresponding mechanisms in the young rabbit are intact some days before the arterial pressure has attained a pressure sufficient to stimulate them.

There is some evidence to show that a measure of sympathetic control may also exist in the sheep's foetus late in pregnancy ; at all events, the classical demonstration of Cyon may be obtained, namely, that of lowering of arterial pressure

without cardiac slowing, on stimulation of the central end of one vagus, with the other vagus cut. It may well be that though the sympathetic is capable of action when stimulated, it is not actually exerting any vaso-motor tone in the uterine sensory vacuum.

In the sheep foetus at 126 days it is possible by exerting pressure behind the angle of the jaw, i.e. just over the carotid sinus, to slow the heart without diminishing the quantity of blood which passes through it (see Fig. 17. 6).

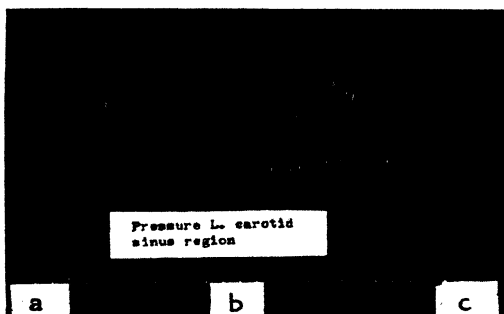


Fig. 17. 6—Slowing of heart by pressure behind the ear ; sheep foetus 126 days. Cardiometer tracing. (From Barcroft and Torrens, 1946.)

The output of the foetal heart.

The output of the heart in the goat has been measured cardiometrically by Barcroft, Flexner and McClurkin (1934). Their figures average approximately 150 c.c. per kilo of foetus per minute and are probably too low. More recent figures obtained by Barcroft and Torrens (1946) are as shown in Table IV. After 95 days it seems probable that the output of the heart though increasing absolutely is a decreasing quantity relatively to the body-weight. In discussing oxygen consumption of the body we shall see that it, too, falls per kilo of body-weight, the principal reason for which is that at the end of gestation there is a great increase in the weight of relatively inert material such as the calcium phosphate in bone, the blood, etc. The quantity of blood with which the heart succeeds in dealing is very striking ; 350 c.c. per kilo of body-weight, compared to the performance of the human body, corresponds to what the human heart would put out if the body were taking active, but not absolutely exhausting, exercise. The amount of work being done in the two cases must be modified by considerations of the pressure against which the blood is being expelled. In man the pressures are very different in the aorta and pulmonary artery respectively ; in the foetus they are the same in both vessels, Pohlman (1909), Hamilton, Woodbury

TABLE IV

Sheep.	Weight, kilos.	Foetal age, days.	Blood flow through heart. c.c.	
			per min.	per kilo per min.
685	0·57	95	225	395
683	0·96	106	345	360
686	1·05	108	410	390
682	2·20	120	640	295
708	2·40	123	501	210
689	1·64	129	380	235
649	2·81	136	800	285
709	4·10	141	770	190

and Woods (1937). In the sheep this pressure at term is much lower than that in the human aorta, but higher than that in the pulmonary artery.

V. THE BLOOD-FLOW THROUGH THE PLACENTA

The placenta has been described as an arterio-venous anastomosis, Burwell (1938). If that phrase is intended to signify that the placenta is an organ through which a large quantity of blood flows and which presents a very low resistance, the phrase is justified. It need scarcely be emphasised that the placenta with its complicated functions and capillary network is something very different from a mere by-pass for blood.

The volume of blood which traverses the placenta per minute has been measured by Barcroft and Torrens in the sheep by a method due to Brodie. The foetus is put in a plethysmograph: when the writing-point indicates a constant level, the umbilical veins are suddenly occluded, the level of the tracing drops.

TABLE V

Sheep.	Weight, kilos.	Foetal age (days).	Blood Flow through Placenta.		O ₂ difference between umb. art. and umb. vein, c.c. per 100 c.c. blood.	Oxygen used by foetus.	
			c.c. per minute.	per kilo of foetus.		c.c. per minute.	c.c. per kilo per minute.
685	0·57	95	113	198	6·3	7·1	12·5
683	0·96	106	173	180	5·3	9·2	9·6
686	1·05	108	205	185	4·05	8·1	7·5
682	2·20	120	320	148	—	—	—
708	2·40	123	251	105	3·7	9·3	3·9
689	1·64	129	190	118	—	—	—
649	2·81	136	400	143	3·6	14·4	6·6
709	4·10	141	385	95	5·7	22·0	5·4

At first the drop, in relation to time, is linear, later it tails off. During the linear period it is assumed that the blood from the foetus is flowing into the placenta at a constant rate, which is the rate at which it was flowing *through* the placenta previous to the occlusion.

The results of these authors are as yet incomplete in detail, but they indicate that the amount of blood which traverses the placenta is of the general order of half of that leaving the heart. It is not as yet clear how this proportion is influenced by the age of the foetus or by other circumstances, but accepting it as a rough generalisation and applying it to the figures in Table IV, we get Table V.

In the sheep the blood-flow through the placenta has been computed directly by Barcroft, Kennedy and Mason (1940). Their method depended on measuring (1) the total amount of oxygen being used by the foetus and dividing this by the oxygen-difference per c.c. of blood as between the blood in the umbilical artery and that in the umbilical vein. The results are given in Table VI. The flows up to 127 days are substantially smaller than those given in Table V.

TABLE VI

Sheep.	Foetal age, days.	Weight, kilos	Blood Flow through Placenta.	
			Total.	Per kilo per min.
259	111	1.20	125	96
325	126	3.00	219	73
327	127	2.85	167	57
347	129	2.75	600	218
274	137	3.85	565	147
320	138	3.65	285	78
351	152	2.80	300	107

Comparing the data in Table V and VI, the flows per kilo of foetus per minute in Table V show a gradual decline as the foetal age increases and are much more regular than those in Table VI. The principal difference is at the earlier age.

VI. THE OXYGEN CONSUMPTION OF THE FOETUS

Three direct methods of measuring the oxygen consumption of the foetus exist, as well as several indirect methods. The direct ones are as shown in Table VII

(1) In terms of tissue slices. Sample tissue slices are taken from the body of the freshly killed foetus, or at least from such organs as are large enough to make an appreciable contribution to the whole. The oxygen uptake of these tissues is obtained in c.mm. of O_2 per mg. dry weight per hour; knowing these figures (Table VII) and combining them with the water content of each tissue and the proportionate weight of each tissue to that of the body, an estimate can be made of the oxygen used per kilo of body-weight per hour. The figures come out as shown in Table VIII.

TABLE VII

OXYGEN UPTAKE OF CERTAIN TISSUES OF SHEEP FOETUS AT DIFFERENT AGES, AND ONE LAMB AND ONE ADULT SHEEP, EXPRESSED AS CU.MM./MG. DRY WT./HOUR.

Tis	Foetal age (days).					Lamb, 6 days.	Adult.
	78	99	130	144			
Skin .	3.7	2.0	3.0	2.8	2.3	2.2	1.2
Muscle .	2.4	3.1	2.0	1.0	0.7	0.6	1.2
Cartilage .	3.1	2.4	0.8	0.6	0.8	0.9	0.3
Bone .	—	0.1	0.1	0.1	0.1	0.05	0
Liver .	10.8	7.5	6.8	6.4	7.3	5.8	3.6
Intestine .	—	—	—	5.2	8.4	7.5	5.6
Stomach .	—	4.7	2.1	4.3	4.4	4.9	0.9
Lung .	2.3	2.2	3.5	4.3	2.8	5.2	3.0
Brain .	—	3.4	6.1	5.8	8.3	13.6	2.9
Blood .	—	0.2	0.2	0.2	0.1	0.2	0.3
Kidney .	8.7	—	—	—	—	—	—

TABLE VIII

ACTUAL UPTAKE OF WHOLE TISSUES PER KILO BODY-WEIGHT IN C.C. PER MIN.

AGE—days.	73	99	130	144	Lamb.	Adult.
Tissue .						
Skin .	0.33	0.40	0.76	1.22	0.92	1.03
Muscle .	1.92	1.75	1.35	0.73	0.54	0.59
Skeleton .	2.44	2.20	0.59	0.40	0.49	0.55
Liver .	2.20	1.10	0.85	0.79	0.72	0.47
Gut .	—	0.32	0.10	0.27	0.39	0.57
Lung .	0.15	0.19	0.29	0.27	0.21	0.27
Blood .	—	—	0.08	0.06	0.02	0.07
Brain .	—	0.27	0.25	0.20	0.26	0.46
Kidney .	0.21	—	—	—	—	—
Total	7.91	6.84	4.24	4.24	4.0	4.23

The parts of the body unaccounted for are assumed to have the same metabolism on the average as those accounted for.

(2) The reduction method of Barcroft, Kennedy and Mason (1940) ; the figures are arrived at in the following way : The umbilical cord is clamped so that the

only source of oxygen supply for the foetus was its own blood. Over a period amounting to an appreciable fraction of a minute the fall in the oxygen of the carotid blood was linear. This fall in c.c. of oxygen per minute multiplied by the known blood volume gave the oxygen utilisation per minute. The results are shown in Table IX.

TABLE IX

Sheep.	Foetal age, days.	Weight, kilos.	Oxygen consumed.	
			c.c. per min.	c.c. per kilo per min.
259	111	1.20	4.6	3.8
325	126	3.00	12.3	4.1
326	127	2.85	11.2	3.9
347	129	2.75	25.2	8.1
274	137	3.85	20.0	5.2
320	138	3.65	15.5	4.2
351	152	2.80	16.4	4.3

(3) The third direct method of measuring the oxygen consumption of the foetus is that of Barcroft and Torrens (1946). The foetus is put in a plethysmograph. The rate of blood-flow through the placenta is measured, then samples of blood are taken from an umbilical artery and vein and the "oxygen difference" so obtained. The oxygen difference multiplied by the blood-flow gives the oxygen consumption. Barcroft and Torrens's observations are as yet incomplete in detail, but they are indicated in Fig. 17.7 and denoted thus ⊕

Comparing the results from these three methods, we may say—

(1) that after 112 days at which the oxygen consumption by the tissue-slice method has settled down to about 4 c.c. per kilo per minute, the agreement between that method and the reduction method is better than might have been expected. If the tissue slices are regarded as representing absolutely basal conditions, the foetuses on which Barcroft, Kennedy and Mason's results were obtained must have been reduced in every case except one to nearly that state.

(2) The results obtained by Barcroft and Torrens show, as is natural, an appreciably higher degree of metabolism corresponding to the greater degree of muscular tone which they exhibited.

(3) Basally, after 112 days the oxygen consumption of the foetus is 4 c.c. per kilo per minute; actually, it exceeds this in proportion to the degree of activity which its organs display. Before 112 days it is impossible to say much.

Considering the probable pitfalls in the method used, the results have come out with remarkable regularity. It is of interest to compare them with some others: for instance, many observations have been made on mature sheep with the respiration calorimeter.

The results shown in Table X on sheep which were under basal conditions in the sense that the sheep had not been fed for 48 hours.

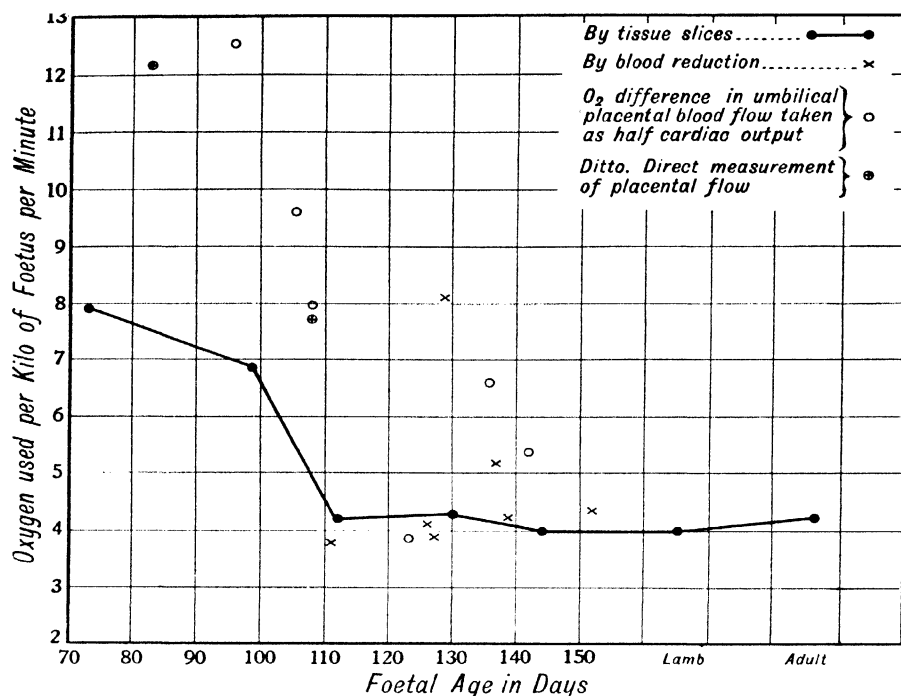


Fig. 17. 7—Estimates of oxygen used per kilo of foetus per minute at successive foetal ages.

TABLE X

OXYGEN UPTAKE FOR SHEEP FROM WHICH FOOD HAS BEEN WITHHELD FOR 24 HOURS
(*Carlyle, 1944*).

Sheep.	Weight, kilos.	O ₂ uptake per 24 hours.	O ₂ uptake per kilo/min.
770/1	45.5	297	4.5
769/2	43.2	252	4.1
	36.3	155	3.0
	31.8	141	3.1
	48.6	283	4.1
	43.2	219	3.5
	36.3	197	3.8
	38.6	188	3.4

The value computed from data quoted from Dukes (1942) (28 calories per kilo per day and an R.Q. of 0.9) is 3.97 for sheep not starved.

There seems little doubt that the oxygen consumption of the pregnant mother (including that of the foetuses) is greater proportionately to her weight (including

the weights of the foetus) than her oxygen consumption would be if she were not pregnant. The oxygen consumption of the pregnant woman per square metre of her body surface is also greater than that of the same woman when she is not pregnant. There is a considerable literature centering around the relation of the oxygen consumption of the non-pregnant mother per square metre of surface to that of the pregnant mother per square metre of her body surface plus the body surfaces of the foetuses. It may be a personal view, but the comparison seems to the present author to lack point, since the foetuses have no surfaces which dissipate heat to the external air. The foetuses are, from the point of view of heat loss, merely an organ of the mother. It would seem more fruitful to forget about the foetal surfaces and to consider the underlying causes of the mother's rise in metabolic rate from the point of view of the metabolic requirements of her offspring. Nevertheless, calorimetric experiments on women have a practical value in so far as they indicate the extra number of calories which pregnancy demands.

For man, Haselhorst and Stromberger (1932) give the oxygen used by the foetus approaching term as 1.25 c.c. per kilo per minute, and after delivery as 1.5 c.c. These figures, it will be noted, are much lower than those for the sheep and goat and are probably open to revision, but that they should have been obtained at all is a matter for congratulation.

VII. CIRCUIT TIME

Haselhorst and Stromberger (1932) injected red pigment into one of the umbilical vessels and noted the time which elapsed before the pigment appeared in samples taken from the cord. The earliest appearance of the red pigment was 50 seconds after the injection. In the cases in which sixty seconds was allowed after the injection, the pigment appeared faintly (+) in nine cases and markedly (++) in one; it was not till 120 seconds that the pigment appeared richly (+++).

In the light of what we now know, but which was not known when Haselhorst and Stromberger carried out their experiment, the pigment may have gone the circuit of the body by very various routes. That which appeared first probably took the *via dextra* on arrival at the heart and, traversing the ductus arteriosus and dorsal aorta, arrived in the minimal time in the umbilical vessels. The general mass of pigment, taking up to 120 seconds to make the circuit, probably took the *via sinistra* and made the circuit of the head before reaching the umbilical cord.

The circuit time in animals for the heart-placental circuit has not been adequately investigated. In sheep, judged by X-ray cinematograph technique, in a single experiment the opaque material travelled from the top of the aorta to the vena cava in 12 seconds (Barclay *et al.* 1944); add to this a second (the time of three beats) for the passage through the heart, and the time for traversing the circuit by the *via dextra* would be, say, 15–17 seconds, or one-third of that taken in Haselhorst and Stromberger's tests. As the sheep foetus and the baby are almost the same size, one conclusion would be that the circulation in the "human" after a normal delivery was rather feeble: another, that the peripheral circulation presented a much higher resistance in the babies.

Some other times may be mentioned : that required for blood to pass from the pulmonary artery to the pulmonary vein, is in the sheep foetus, about three seconds (Barclay, Barcroft, Barron, Franklin and Prichard, 1942) and about half that after the closure of the ductus arteriosus. The time given for the circuit brachiocephalic artery to anterior caval channel is 5-8 seconds. This last measurement is of interest in relation to Haselhorst and Stromberger's figures : for again allowing two or three seconds for the transit of the heart and the routes of the great vessels, it might be inferred that blood reaching the heart from the umbilical vein and taking the route by the *via sinistra* would take about ten seconds longer to return to the placenta than had it taken the *via dextra*—i.e. it would have made the complete circuit, both of the placental capillaries and those in the head in, say, 27 seconds or less than half a minute. Again, the question arises—why should this time be so much shorter than that indicated by Haselhorst and Stromberger?

VIII. BLOOD PRESSURE

Arterial pressure.

The following figures have been given for arterial pressure in foetuses at or near term :—

TABLE XI

Author.	Animal.	Arterial pressure at term mm. Hg.	Vessel.
Ribemont . . .	Man	64	Umbilical artery
Setz . . .		73	
Haselhorst . . .		76-110	
Cohnstein and Zuntz . . .	Sheep	83	
Barcroft . . .	"	68-78	Carotid artery
Schlossmann . .	Goat	50-60	
Clark . . .	Cat	25-30	
	Dog	50/0	Left ventricle
Hamilton, Wood- bury and Woods	"	50/0	Right "
	Rabbit	21/0	Left "
	"	21/0	Right "
Corey . . .	Rat	10 (20-24)	

The results in Table XI give very divergent figures for foetal arterial pressure at term, but in a very general way the forms which are least developed at birth have the lowest arterial pressures : which suggests a gradual rise in arterial pressure as intra-uterine development proceeds. The only extensive studies have been made by Barcroft and his colleagues (1945) over several years, those prior to 1940 in association with Barron and Forsham. These observations go far to explain the divergences noted above.

¹ Taken from *Researches on prenatal Life*. Barcroft (1946).

(1) If due precautions are taken the pressure in the umbilical artery is within about 5 mm. of the following :—

TABLE XII

Foetal age (days)	40	60	80	100	120	140	147
Pressure in umbilical artery mm. Hg.	5	20	26	32	38	45	50

(2) In the later stages of gestation the pressure in the umbilical artery is several millimetres below that in the carotid.

(3) If the procedure used by some observers is followed, namely, if the foetus is delivered into a saline bath, a cannula being inserted into the carotid or the femoral artery of the sheep, the arterial pressure towards the end of pregnancy is very much higher than the above figures indicate, being in the region of 70–80 mm. Hg, whilst that of the born lamb is 100 mm. or thereabouts.

The cause of the discrepancy between the arterial pressures at term as measured by a needle slipped into the umbilical artery (say 50 mm.) and by a mercurial manometer (say 75 mm.) is not fully known. As noted above, a discrepancy of a couple of millimetres is attributable to the fact that the carotid pressure is in any case higher than the umbilical pressure—a fact probably due to the low peripheral resistance of the placenta as compared with the head. Again, when the needle is used the flow of blood in the vessel is relatively unimpeded; when the vessel is cannulated it is tied peripherally to the cannula. These small discrepancies mount up, but even when they are added together, a large margin is left between the femoral arterial pressure as measured by a mercurial manometer and the umbilical arterial pressure as measured by the needle. Two suggestions may be put forward to explain the difference :

(1) We have seen that exposure of the foetus to the experimental conditions contingent on cannulation of the carotid or femoral removes, or greatly lessens, the effect of the depressor nerve on the pulse. It is possible, therefore, that the same conditions may remove the effect of the depressive nerves on the vaso-motor system.

(2) The whole process of cannulisation, including the necessary exposure of the skin, must involve a field of general sensory stimulation, which in turn may have a powerful effect upon the sympathetic system with consequent rise of vaso-motor tone and arterial pressure. In other words, the foetus in utero is probably in something approaching a sensory vacuum—a vacuum which is abolished by the surgical operative procedure. The reaction of the sympathetic system to general sensation has been shown by Adrian, whilst, of course, the fact that stimulation of the sensory fibres of the sciatic caused a rise of arterial pressure is a very old experiment.

Venous pressure.

The only vessel concerning which we have measurements is the umbilical vein. Formerly, and on the basis of the work of Cohnstein and Zuntz (1884), this was supposed to be very high, averaging 27 mm. Hg. Actually, this high

result seems to depend upon undue interference with the circulation. The following are average figures given by the needle technique :—

TABLE XIII

Approximate foetal age (days)	60	75	88	115	140
Pressure in stream umbilical vein mm. Hg	8	8	8	9	(14) *

Unlike the pressure in the umbilical artery, that in the vein remains very constant throughout most of foetal life ; however, it undergoes considerable variation at the very end. It is higher than the normal pressures in the caval systems of mature animals, but lower than the pressures usually registered in the portal vein. It will be recollected that the major portion of the blood in the umbilical vein traverses the vessels of the liver, the minor part only traversing the ductus venosus.

More interesting than the pressure in either the umbilical artery or the umbilical vein is the difference between the two, i.e. the head of pressure available for the propulsion of blood through the placenta. This increases as gestation proceeds, being about 12 mm. at 60 days and 30 or more at term. In other words, the increase is two-and-a-half fold, or perhaps more (Fig. 17. 8).

IX. THE OXYGEN CAPACITY OF FOETAL BLOOD

For the present purpose oxygen capacity will be taken as synonymous with haemoglobin value, it being assumed that 1 gram of haemoglobin unites with 1.34 c.c. of oxygen.

The oxygen capacity of blood rises throughout foetal life ; this has been shown by Elliott, Hall and Huggett (1934) for the goat and by Wintrobe and Shumacker (1935, 1936), for the pig, rat, rabbit, cat, dog and man.

The most complete data are, however, those obtained on the sheep by Barcroft and his colleagues. When the oxygen capacity is plotted against the foetal age, a curve of a mildly sigmoid character is obtained as the average of 134 cases, the rise in haemoglobin value being rapid between the 35th and 60th days, slow between the 60th and 120th, and slightly more rapid towards the end. But a curve based simply on average values does not bring out some of the material facts. These are—

(1) There is a rapid rise in the oxygen capacity of the blood during the first 90 days or so of foetal life.

(2) After that the rise is more gradual.

(3) In the last twenty days the rise, on the average, becomes slightly more rapid.

(4) The distinguishing feature, however, of the third period is the great individual variations which occur. The lowest values are much below those observed in the previous weeks, and rather suggest the onset of some form of

* This figure is of doubtful validity, being the mean of only two observations, 22 and 9 mm.

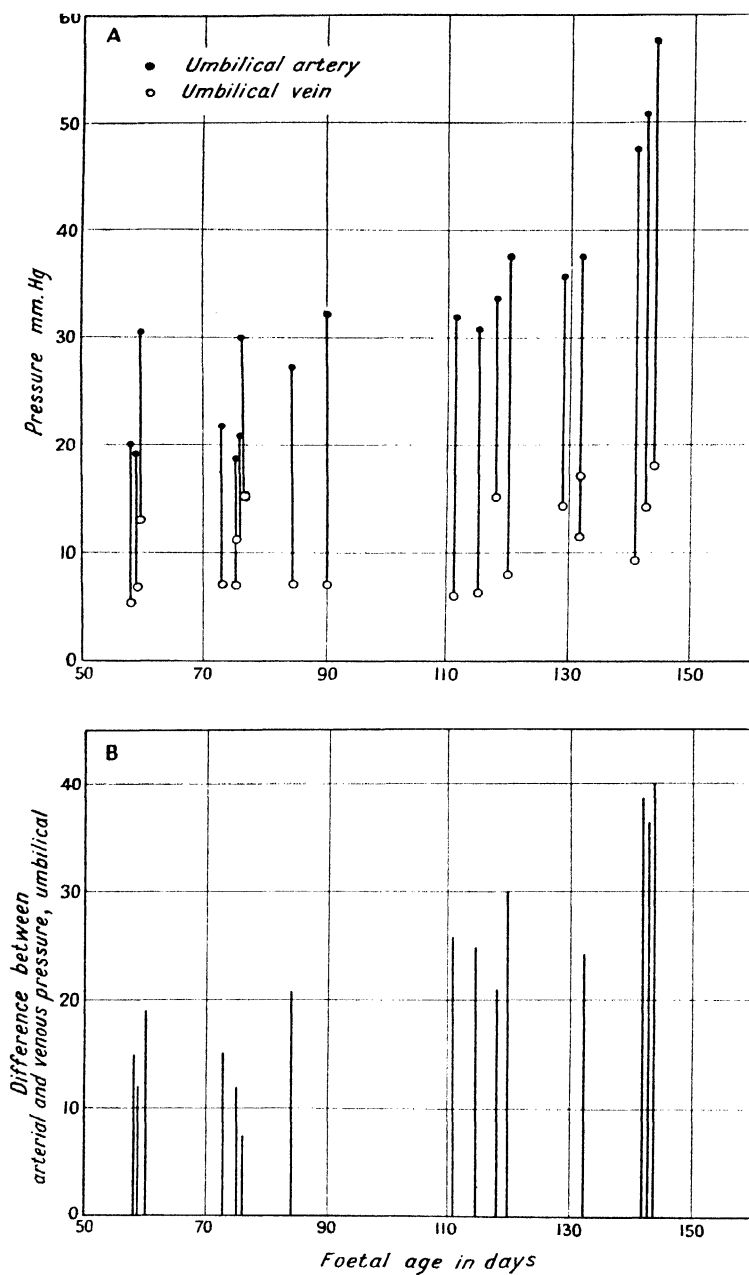


Fig. 17.8—A. Blood pressure in umbilical vessels considered in respect of foetal age. B. Difference of pressure as between umbilical artery and vein considered in respect of foetal age. (From Barcroft, 1946.)

anaemia. This, if it occurs, might be due either to malnutrition or to some hereditary cause. In man such an anaemia is known to exist, and cases of heterogenous twins have been recorded in which one twin has a normal haemoglobin value, whilst the other has an anaemia due to the action of an Rh factor.

(5) If, indeed, the low figures which appear just before birth are due to an abnormal condition, they may be struck out of the normal picture. In that case the normal curve would rise more sharply at the end of pregnancy, as might naturally be expected as the result of the stringency of the oxygen supply at the disposal of the foetus.

(6) In some species the oxygen capacity of the blood at the end of foetal life rises to a level considerably higher than that of the adult of the same species. In other species that is not the case. Wintrobe and Shumacker consider that the more primitive condition in which the foetus is born, the lower is likely to be the haemoglobin capacity of its blood.

(7) In any case, there is frequently a temporary fall in the oxygen capacity of the blood following on birth.

X. THE BLOOD PICTURE

The Erythrocytes

The erythrocyte blood picture has been worked out in great detail by Wintrobe and Shumacker (1935). I cannot do better than give their own summary of findings :

"1. Determinations of the erythrocyte count, mean corpuscular volume, mean diameter and proportion of nucleated red corpuscles and reticulocytes in the blood of fetuses and newborn of man, the pig, rabbit and rat, are graphically recorded.

2. It is shown that in the very young fetuses of the species examined, the erythrocyte counts are very low and the red corpuscles are very large when compared with the values for red cell count and size in the normal adult of each species. As the fetus develops the erythrocyte count rises, and the red corpuscles become smaller. The proportion of nucleated red corpuscles decreases very rapidly, while the percentage of reticulocytes diminishes more gradually.

3. In all of the species examined, some macrocytosis was found to be still present at birth ; and in the rat, rabbit, pig, cat and dog the erythrocyte count was lower than that of the adult. In man, the erythrocyte count is approximately normal at birth. In the newborn dogs examined, the counts were substantially below the adult values and in the newborn rats they were approximately one-third of those of the mature rat."

These observations suggest that the macrocytosis of the newborn represents a final stage in the normal development of the blood.

"4. Comparison is made between the development of the blood in the fetus and the changes which occur in the blood of cases of pernicious anemia in response to liver therapy.

5. The blood of the fetus resembles in many respects that of cases of pernicious anemia which are being subjected to an effective continuous and extremely potent stimulus to blood formation."

The considerable literature will also be found in their paper.

Concerning the white blood corpuscles, there is little to be said ; so far as cats are concerned, an estimate of them is given in Fig. 17. 9.

XI. THE PLASMA

A discussion of the constitution of the plasma in relation to that of the mother is to be found in *Biochemistry and Morphogenesis* (Needham, 1942) where it is summarised in the following words : " It may in general be said that the maternal blood is richer in total solids, proteins, total P, lipoid P, neutral fat, cholesterol and glucose ; while the foetal blood is richer in non-protein N, free amino-N, in organic P and calcium. There are, however, certain exceptions to this ; for example, in ungulates (epithelio-chorial placentas) the blood sugar is higher in

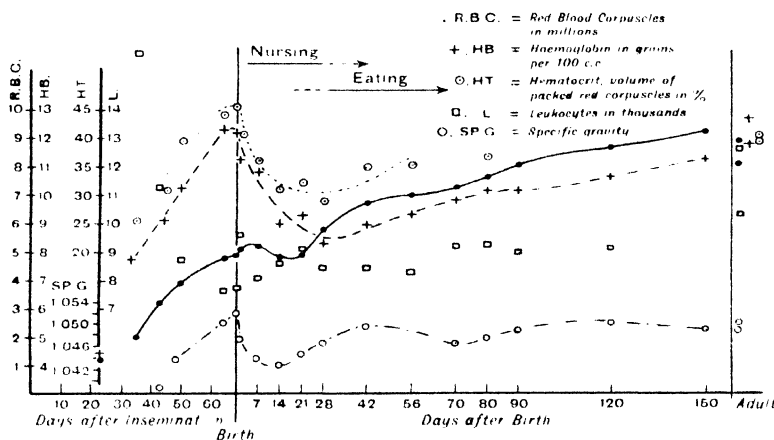


Fig. 17. 9—Number of red blood corpuscles (•) in millions, leukocytes (□) in thousands, volume (haematocrit) of packed red corpuscles (○) in per cent., amount of haemoglobin (+) in grammes per 100 cc. and specific gravity (○) of the blood of cats during prenatal and postnatal life until maturity. Adult values for males are higher than for females in all except specific gravity. Each point represents an average. (From Windle, 1940.)

the foetal than in the maternal circulation. The concentrations of urea, uric acid, creatin, creatinine chloride, etc., are usually the same." It seems only necessary to take up a few points which have arisen since 1942.

This is not the whole story, for the paragraph just quoted gives the impression that the plasmas of the foetus and mother respectively contain the same substances and that the difference between them is purely a quantitative one. That, however, would be an understatement ; the maternal plasma contains constituents which, though abundant in it, are only found to a vestigial extent in the plasma of the foetus and, conversely, there are constituents in the foetal plasma which scarcely exist in that of the mother.

Proteins.

In the dog (Clark and Holling, 1931), the sheep, the horse (McCarthy, 1938) and man (Bridge, Cohen and McNair Scott, 1941) the percentage of protein in foetal blood is less than in maternal.

The following figures are given for man and sheep respectively :-

TABLE XIV

Species.	Percentage of Protein in Plasma.			Osmotic Pressure mm. Hg.	
	Total.	Albumen.	Globulin.	0° C observed.	37° C calculated.
<i>Man.</i> (Bridge, Cohen, & McNair Scott)					
Premature babies	4.7	3.58	3.18	—	—
Pregnant women	5.8	3.72	2.05	—	—
Adults	6.8	4.50	2.50	—	—
<i>Sheep.</i> (Serum)(McCarthy)					
A (Foetus)	4.89	3.04	1.85	19.6	22.3
(Mother)	7.98	2.96	5.02	22.5	25.5
B (Foetus)	4.34	2.74	1.60	16.5	18.7
(Mother)	6.61	2.45	4.16	17.3	19.6

Clark and Holling (1931) observed that in the period shortly after birth, "as the protein content increases, the osmotic equivalent of each gram of protein also increases."

Fig. 17. 10 shows the relative osmotic pressure of the serum protein in the ewe and mature foetus respectively.

Clearly the mean molecular weight of the maternal proteins must be greater than those of the foetus. In the calf there is a simple explanation, namely, the considerable quantity of a globulin (MW 57,000) called fetuin, present in the foetal but not in the maternal plasma (Pedersen, 1944).

On the other hand, the foetal plasma seems to lack protein found in the mother. The fact was discovered by Howe (1921) on the

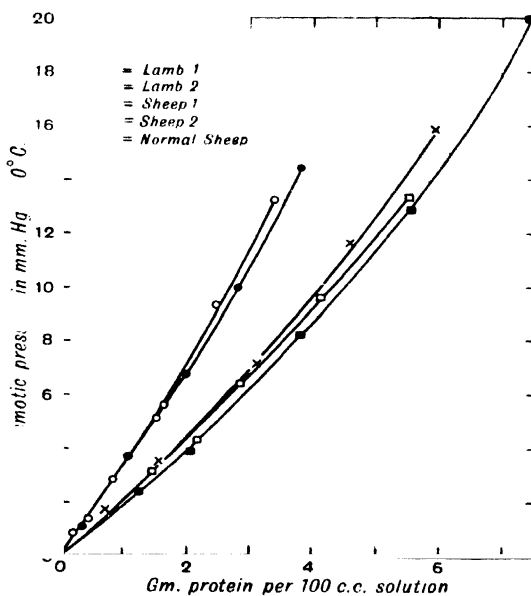


Fig. 17. 10—Osmotic pressure of proteins in sera of sheep and newborn lambs. (From McCarthy, 1938.)

basis of "salting out" experiments. More recently the ground has been covered by Jameson, Alvarez-Tostado and Sartor (1942) who used kataphoretic methods. They give the following figures :—

TABLE XV
MOTILITY DATA OF CALF SERUM

(Cms. per sec. per voltmeter $\times 105$, buffer 0.15 molar NaCl $\times$ phosphate 0.02 molar, pH = 7.7.)

Time.	Globulin.			Albumen.
	β			
New-born	—	—	4.66	6.53
18 hours	—	3.49	5.34	7.38
36 hours	2.11	3.80	5.79	7.58
3 days	2.30	3.69	5.17	6.58
5 days	1.86	3.26	4.55	6.28
2 years	1.74	3.43	4.65	6.55

This acquisition of globulins γ and β within the first few days of pregnancy is attributed to the ingestion of protein from the colostrum of the mother. The γ globulin more or less covering the euglobulin of the salting-out method is regarded as important (Orcutt and Howe, 1922) because the agglutinins for *Bacillus abortus* are associated with it.

Reverting to the fact that the osmotic pressure of the plasma protein of the mother is as great as, if not greater than, that of the foetus, the question arises what force (if I may be forgiven the word) is responsible for the passage of water from the maternal to the foetal blood? Actually, the net number of water molecules which traverse the placental barrier is greater than that of any other substance. Were the osmotic pressure of the proteins of the foetal plasma greater than that of the maternal plasma, some basis would exist for the supposition that the passage of water to the foetus was an osmotic effect; but this is not the case, nor is the mystery simply one which affects sheep's blood; if so, we might find a solution in the quantity of fructose (see below) present. Indeed, the difficulty was first pointed out by Clark and Holling (1931), the subjects investigated being puppies.

Carbohydrates.

It is currently said that the percentage of blood sugar in the blood of the mother exceeds that in the foetal blood, except in the case of ruminants, in which the position is reversed. The excess of "blood sugar" in the foetal blood of the sheep is accounted for by the considerable quantities of fructose present in the plasma of the foetus. Fructose occurs only in negligible quantities in that of the mother. The observation was first made by Cole and Hitchcock using a micromethod,

which covers any other ketose in the plasma, and has been confirmed by Bacon and Bell (1946) who have isolated fructose.

The following is a sample of Cole and Hitchcock's (1946) results, which cover foetal ages from 72 days onwards.

To give a single example :—

TABLE XVI
SUBSTANCES EXPRESSED IN MG. HEXOSE PER 100 C.C. BLOOD

Serial number and age.	Vessel.	Blood Sugar.	Fructose.	Difference presumably preponderantly glucose.
623 76 days	Umbilical vein	149	103	
	Umbilical Artery	143	100	
	Mean	147	102	45
	Uterine Artery	88	1	
	Uterine Vein	110	1	
	Mean	99	1	98

Taking the above figure : (1) Two-thirds of the blood sugar in this particular foetus is fructose, whilst the amount of fructose in the maternal blood is only 1 mg. per cent. (2) The "glucose" in the maternal blood is about twice that in the foetal blood. (3) There is an appreciable loss of glucose from the maternal blood in its passage through the placenta.

Fats.

The problems relating to fats in the foetal plasma centre for the most part around its passage across the placental barrier and therefore are considered under the placenta. Up to 1942 they are discussed by Needham (1942). Since that date Barcroft and Popják (1944) have made a very careful series of observations on the fats in the foetal and maternal blood in the sheep at successive periods in foetal life.

Fig. 17. 11 indicates that no constituent of the foetal fats has a higher value in the foetal than in the maternal blood, and that for the most part the value in the foetal blood is $1/3$ to $2/3$ that in the maternal. The rabbit, however, gives quite different results.

XII. THE OXYGEN IN THE BLOOD

The oxygen in the blood may be considered in respect of certain vessels, and of these we may take first the umbilical artery.

Fig. 17. 12 gives a general picture of the percentage saturations of the bloods in the umbilical artery and vein respectively in the sheep, the only animal in which the data have been observed systematically over the greater part of the gestation period.

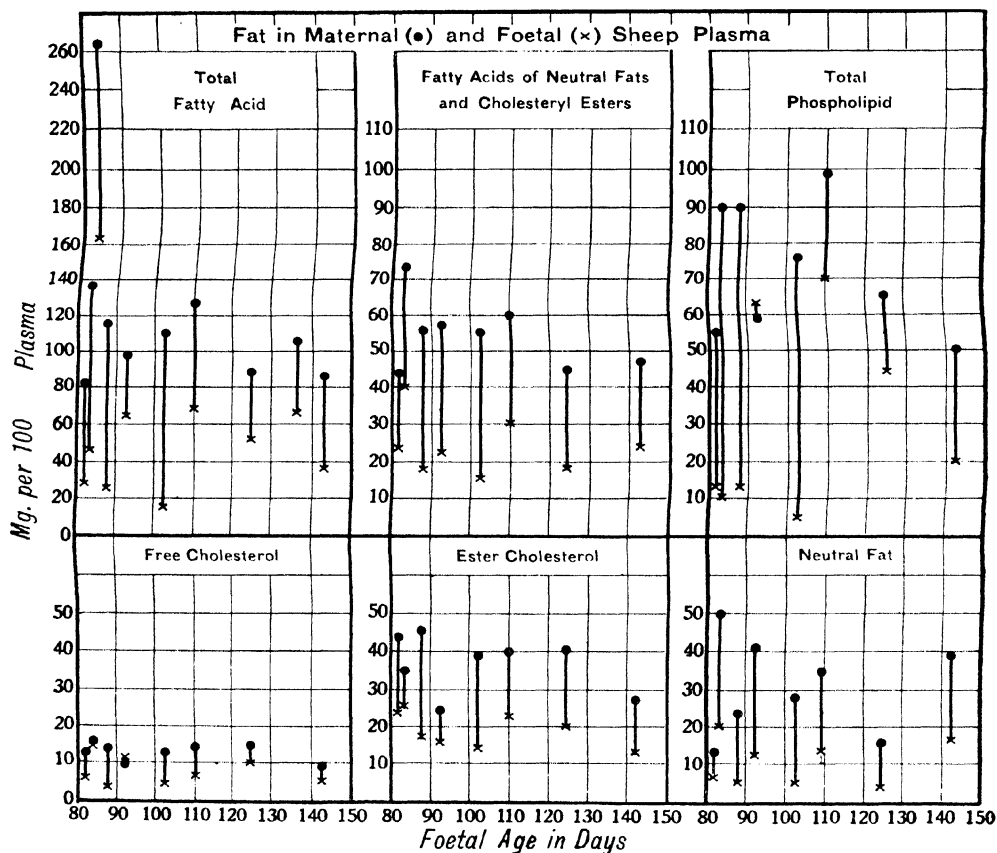


Fig. 17. 11—Fats in maternal and foetal blood (sheep).
(From Barcroft and Popják, 1944.)

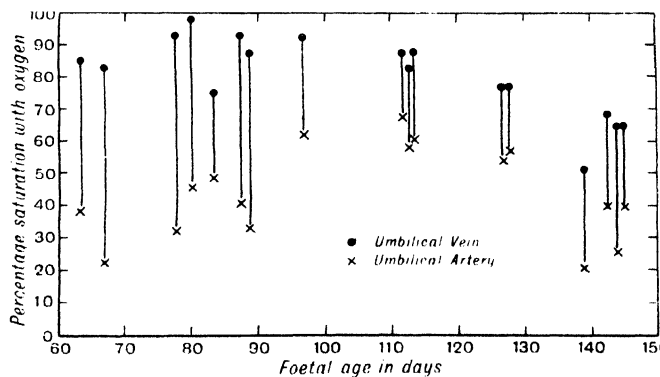


Fig. 17. 12—Percentage saturation of blood in umbilical vessels during foetal life.
(From Barcroft, Kennedy and Mason, 1940.)

The general features are :

In the umbilical vein the oxygen saturation is high : 80-90 per cent. in most cases earlier than 120 days, after which it declines. At term the observations are more variable than at other times, but are usually in the region of 65 per cent.

In the umbilical artery the saturations are low at 60-70 days ; in the vicinity of 90-100 days they suddenly rise to about 60 per cent. and then drop to values at term of 20-40 per cent.

The "oxygen difference" is very great at the 60-day stage, perhaps 60-70 per cent. saturation ; it is relatively small at the 110-120-day stage, about 20 per cent. saturation ; and increases again at term, being on the average about 30 per cent. saturation.

In terms of actual quantities of oxygen in the blood :

1. In the umbilical vein the oxygen is low in the 60-70-day stage ; after 90 days, however, it remains extraordinarily constant in most cases at a level of 11-13 volumes per cent.

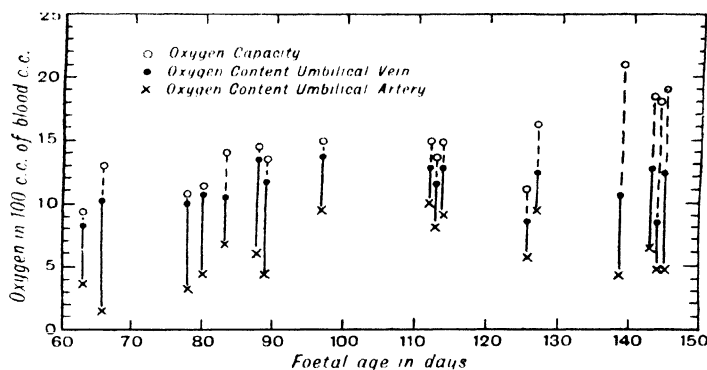


Fig. 17. 3—The oxygen capacity and oxygen content of blood in umbilical vessels during foetal life. (From Barcroft, Kennedy and Mason, 1940.)

2. In the umbilical artery there is very little oxygen in the blood at the 60-70-day stage ; at about 100 to 110 days it reaches a maximum of about 9 volumes per cent. and then drops to about 5 volumes per cent. at term.

3. The oxygen difference is at first considerable ; it reaches its minimal value at the 110-120-day stage, about 3 volumes per cent., and increases again at term, being usually about 6 volumes per cent.

The differences between the picture yielded by the percentage saturations, which are dictated by the conditions of diffusion across the placental barrier and the dissociation curves of the foetal and maternal haemoglobins, and the picture yielded by the absolute quantities of oxygen, are, of course, due to the increasing oxygen capacity of the blood ; this is shown in Fig. 17, 13. The principal effects of this increase are seen as term approaches.

1. In spite of the drop in percentage saturation, the absolute quantity of oxygen in the blood of the umbilical vein is approximately maintained.

2. Though the oxygen utilisation in terms of per cent. saturation between 100 and 140 days only increases in the ratio of 2 : 3, in terms of absolute quantities it increases from 1 : 2. To this we shall return (p. 432).

The dorsal aorta.

The oxygen of the blood in the dorsal aorta is, of course, the same as that in the umbilical vein.

The carotid artery.

The blood in the carotid artery is identical with that in the arch of the aorta. Because the blood in the carotid artery is derived from that in the umbilical vein, plus a certain amount of venous blood from the liver, the carotid blood contains less oxygen than that in the umbilical vein. Likewise, because the blood of the dorsal aorta is a mixture of that from the aortic arch and that from the right heart via the ductus arteriosus, the blood in the carotid will contain more oxygen than that in the umbilical artery. The carotid blood is, therefore,

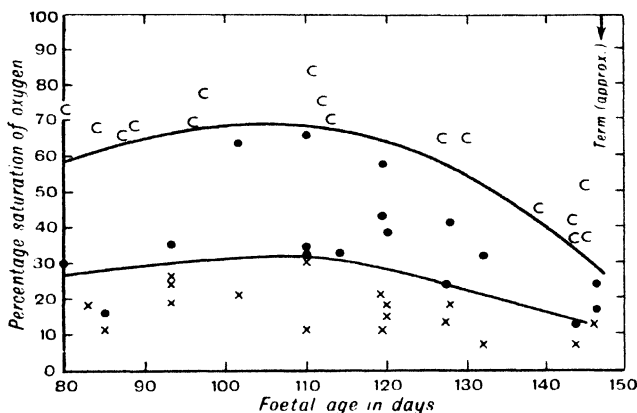


Fig. 17. 14.—Percentage saturation with oxygen of blood of foetal sheep; C in carotid artery; ● in venous sinuses of brain, no respiratory movements occurring; x in venous sinuses of brain when respiratory movements had appeared after pinching cord. (From Barcroft, Barron, Cowie and Forsham, 1940.)

intermediate in composition between the blood of the umbilical artery and that of the umbilical vein. In the earlier stages of gestation the carotid blood contains but little less oxygen than that in the umbilical vein; at term it contains but little more than that in the umbilical artery (Barcroft, Barron, Cowie and Forsham, 1940).

Venous sinuses of the brain.

The oxygen content and percentage saturation of the blood in the venous sinus of the brain is of interest because, taken with the carotid blood, it gives us a picture of the conditions in the brain itself: and this, again, is of interest because if, by pinching the umbilical cord, the oxygen be lowered beyond a certain point, respiration commences. The limiting condition of oxygenation below which respiration occurs is shown in Fig. 17. 14. During the last month the brain becomes definitely less sensitive to asphyxia.

The oxygen dissociation curve.

That the oxygen dissociation curve of the foetal blood was different from that of the maternal blood was first shown by Haselhorst and Stromberger, who worked on human subjects.

Behind this phenomenon in man there is a very remarkable story, which extends to the fish, the chick and the lower mammals. On the foetal side it amounts to this: the earlier the ontological development, the less inflected the curve and the greater the saturation at a given pressure, except at the top of the curve. In other words, the typically embryonic curve, whether it be that of the fish, the frog, the chick, the rabbit, the goat, or man, is that of a haemoglobin adapted to the acquisition of oxygen in the rather exiguous conditions in which the embryo finds itself, whilst the maternal haemoglobin is adapted to the supply of oxygen to the oxygen-hungry tissues of the active organism. This is true not only of the blood but of the haemoglobin in dilute solution. Between the haemoglobin in dilute solution, however, and that in the blood itself there are two stages at which the properties of the dissociation curve may alter: (a) at a

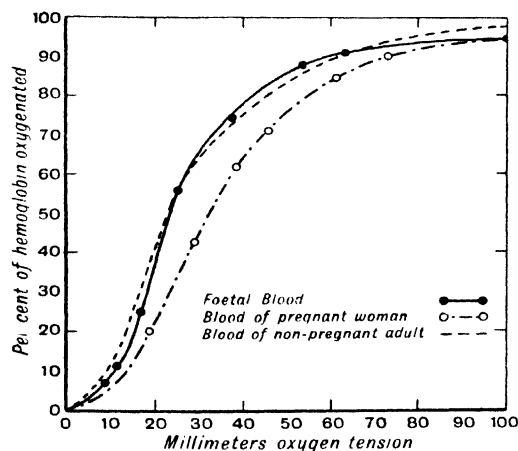


Fig 17. 16—Oxygen dissociation curves of human foetus at birth, pregnant woman, and non-pregnant adult woman. (From Eastman, Geiling and de Lawder, 1933.)

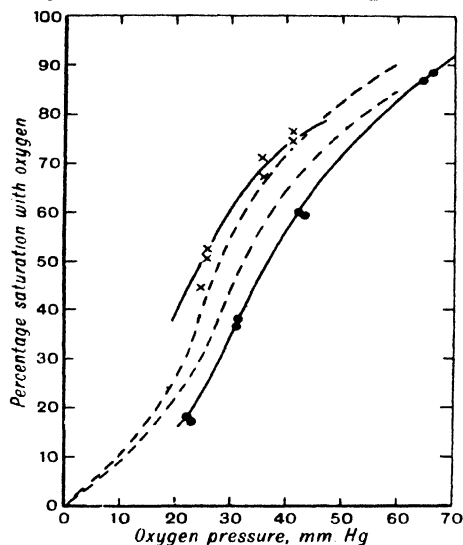


Fig. 17. 15—Oxygen dissociation curves of goat (15 weeks) at 14 mins. and 38° C. CO_2 pressure . . . x foetus ; ● mother. The dotted lines indicate the limits for non-pregnant female goats. (From Barcroft, Elliott, Flexner, Hall, Herkel, McCarthy, McClurkin and Talaat, 1934.)

dilution of about ten per cent. : and () by being packed in the intracorporeal atmosphere.

Comparing the oxygen dissociation curves of the mother and foetus under comparable conditions, the following statements may be made with regard to the parts of the dissociation curve which are physiologically operative :

(1) In blood the haemoglobin of the foetus is more highly saturated at a given oxygen pressure than that of the mother.

(2) In a strong haemoglobin solution the same is true.

(3) In all observed forms except man, the dissociation curve of blood takes up oxygen less readily than that of strong haemoglobin.

(4) Weak haemoglobin solutions take up oxygen more readily than strong ones.

(5) The comparison of the degree of inflation of the various curves is perhaps beyond the scope of this chapter.

The physiological point is that if two bloods, foetal and maternal, of the same percentage saturation are separated by a membrane such as the placental barrier, the foetal blood will acquire oxygen at the expense of the maternal. (Figs. 17. 15 and 16.)

XIII. THE VOLUME OF BLOOD IN THE FOETAL CIRCULATION

Three sets of observations on the foetal blood volume exist (1) by Cohnstein and Zuntz (1884) on rabbits, (2) by Elliott, Hall and Huggett (1934) on goats and (3) by Barcroft and Kennedy (1939) on sheep. In the case of the first and last of these an attempt was made to apportion the distribution of the blood as between the vessel in the actual body of the foetus and those in the foetal vessels of the placenta.

In the Cohnstein and Zuntz records the foetal ages of the rabbits are not recorded, but this can be calculated approximately from the weights which are given; when this is done the same general picture appears as in the case of the sheep, making allowance for the more mature condition of the sheep at birth.

The picture for the sheep is given in Fig. 17. 17b.

The principal features are:

(1) The vascular bed in the placenta has attained a value of 100 c.c. by the hundredth day of gestation. It is doubtful whether this is ever exceeded by much, though there is some evidence of a maximal value at about the 130th day. In the rabbit, also, the volume of the vascular bed at term is no greater than it was some days previously, though there it is possible that a peak existed shortly before term.

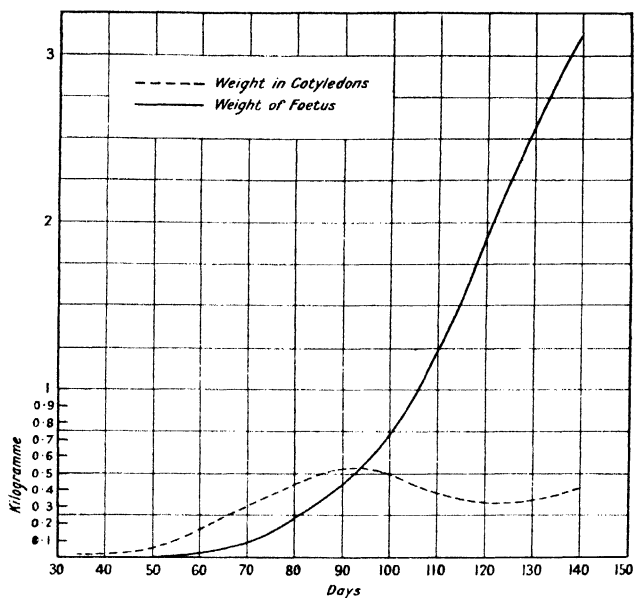
(2) The vascular bed in the body of the foetus, on the other hand, rises continuously as foetal life progresses.

(3) As Elliott, Hall and Huggett point out, of the total quantity of foetal blood, adding that in the placenta to that in the body, the foetal blood bears a fairly constant ratio of about 10 per cent. to the combined weight of the placenta and the foetus. This is not quite true in detail, but the comparison between Fig. 17. 17a and 17b which gives the weights of the placenta and foetus respectively during gestation is a very striking one.

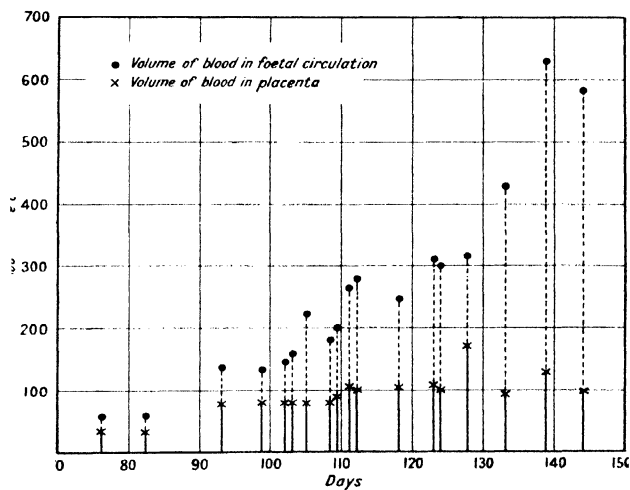
The fate at birth of the blood in the placenta.

From Fig. 17. 17b it appears that one hundred cubic centimetres of foetal blood, or perhaps more, are contained in the vessels of the placenta at term. The question naturally arises, what happens to these at birth? The answer has been attempted by Barcroft and Gotsev (1937) on sheep and by de Marsh, Windle and Alt (1942) on human beings.

The former authors point out that at birth a considerable pressure is set up in the placenta which wrings the blood out of the placental vessels. In four cases in which the blood was collected from the cord it amounted to 75, 83, 80 and 79 c.c. respectively.



(a)



(b)

Fig. 17. 17—(a) Average weights of placental cotyledons and foetus respectively considered in relation to foetal age (sheep). (b) Volume of foetal blood in foetus and placenta respectively considered in relation to foetal age (sheep). (From Barcroft, 1941.)

Barcroft and Gotsev measured the blood transferred to the foetus in the following way :—

(a) The total volume of foetal blood in the foetus and placenta taken together

(V_T) was measured. The cord was then tied and immediately afterwards the volume in the foetus (V_F) was measured.

$V_T - V_F$ gives the blood in the placenta.

(b) A control measurement was made of the blood volume of a control lamb born naturally (V_L).

$V_L - V_F$ gives the amount of blood transferred from the placenta to the foetus. In three experiments the results were as follow, expressed in c.c. of blood per kilogram of lamb.

TABLE XVII

BLOOD VOLUME OF LAMBS OF COMPARABLE SIZE, 3 KILOS $\pm$ 600 GRAMS, IN C.C. PER KILO

Serial Number.	V_T .	V_F .	V_P .	Serial Number.	V_L .
166	213	153	60	188	168
169	209	119	90	241	161
172	172	108	64	246	168
mean	198	127			166

On the means the difference between V_L and V_F is 39 c.c. per kilo of lamb, that being the quantity of blood expressed from the placenta into the foetus at birth.

de Marsh, Windle and Alt (1942) give the blood volume of babies at birth (*a*) in which the cord was tied early and (*b*) those in which it was tied late. Their observations fall into three categories according to the blood volumes of the babies, which vary greatly :—

TABLE XVIII

BLOOD VOLUME C.C. PER KILO

Cord tied.	Low.	Blood volume: Medium.	High.
Early . . .	70-75	90-95	125-130
Late . . .	80-85	100-105	145-150
Difference per cent.	13	11	14

The experiments of de Marsh, Windle and Alt suggest then, that if the ligature of the cord is postponed at birth, about one-seventh of the blood in the foetus is expressed into it from the placenta : whilst those of Barcroft and Gotsev in the lamb suggest that in a lamb normally born, about one-fifth of the blood is wrung from the placenta. The data from both sets of experiments can only be considered as approximations, but they suggest that the amount is considerable.

We may pursue the question further and ask what happens to this blood after its arrival in the lamb or child as the case may be ? A priori, it is difficult to say. Reasons might be imagined for supposing that the lamb required more

blood than the foetus: it is living a more active existence, and, moreover, its lungs have commenced to function. On the other hand, the vessels of the lamb appear to be in a more active state of tone which makes for a smaller vascular bed.

There seems no reason for believing that the lung contains more blood after birth than before it. The iron content of the lung, which may be taken as a rough measure of the red corpuscles, is almost identical as observed by Abel and Windle (1939) working on the cat. This observation has been confirmed on the sheep by Blaschko.

It is, perhaps, beyond the scope of a chapter on foetal life to pursue the fate of the blood further into the period of growth of the new-born animal.

Suffice it, therefore, to say that according to Gotsev:

(a) In the first days after birth the total quantity of haemoglobin in the lamb decreases by about 10–15 grams, which is not far from the amount which the placental blood contributes.

(b) That lambs are born with very different quantities of haemoglobin, per kilo of lamb, in their circulation.

(c) That by the end of a month the haemoglobin has settled to about 12 grams per kilo.

(d) By the end of a month all the lambs had made up the haemoglobin which they had lost.

It is generally held that the haemoglobin which disappears from the blood is broken down and the iron is stored in the liver against the day when fresh supplies of the pigment are required.

This may be the place to attempt some sort of answer to the following question: if, on the one hand, the vascular bed is fixed in volume after the 100th day, and, on the other, the vascular bed of the foetus, the total blood-flow through that bed and the oxygen used by the foetus all increase several-fold during the corresponding period, how does the stereotyped placenta suffice for the ever-growing foetus?

The answer seems to involve one of those examples of physiological integration by which a very large effect is attained by the multiplication of a number of smaller ones.

So far as blood-flow is concerned, it must be remembered that the actual capacity of the placental bed is one thing, the amount of blood which is circulated through that bed is another and will depend principally on the pressure difference between the arterial and venous ends: this we have seen to be perhaps 23 mm. at 110 days and near 40 at term. We have seen also that the arterial pressure in an animal under experimental conditions (as the measured blood-flows are, of course, under experimental conditions) may be 70 mm., giving a pressure difference of about 60 mm.—two-and-a-quarter times that at 110 days. From Table V we see that the actual blood-flow through the placenta per minute, also under experimental conditions, takes from about 200 c.c. to 400 in the same period—a rise of 100 per cent.

The alteration in blood-pressure comes within a measurable distance of accounting for the increase in blood-flow through the placenta, but in order to account for the increase of oxygen uptake, some other factors must be taken into account. Of these the first is the increase in haemoglobin value of the blood. Taking the oxygen capacity of the foetal blood at 110 days as 14·8 vols. per cent.

and that at term as 20 vols. per cent., it follows that each cubic centimetre of blood at term carried $\frac{140}{100}$ per cent. (say 140) times as much haemoglobin as at 110 days. If the quantity of blood increases in the ratio of doubles, then the whole amount of haemoglobin circulated per minute at term will be $\frac{200 \times 140}{100 \times 100}$ times that at

110 days, i.e. as 2.8 is to 1, and the oxygen taken up by the blood might be expected to be increased in the same proportion. Actually, between 110 days and 147 days, so far as may be judged roughly by combining the figures in the last column of Table VI with the average weights of the foetus at 110 days and term respectively, taking the weight at 110 days as 1.25 kilos and the O_2 uptake at 85 c.c. per kilo per minute, the O_2 uptake of the foetus at 110 days would be 10 c.c. per minute, and again taking the weight of the foetus at 147 days to be 3.3 kilos and the oxygen uptake per kilo to be 5.5 c.c. per kilo per minute, the oxygen uptake of the foetus would be 18 c.c. This very rough calculation on all too scanty data does not indicate that the vascular conditions fall short of what is required of them.

The course of the blood-stream.

Regarding the course of the blood-stream in the foetus, it was shown by Pohlman (1909) that the volume of each ventricle of the foetal heart was the same and the pressure at which the blood was put out was the same on the right and left sides. The latter was verified by Hamilton, Woodbury and Woods (1937). Let us assume, therefore, that for every 3 c.c. of blood put out by the right side into the pulmonary trunk, three are put out by the left into the aortic arch. Now let us consider whence come the streams to the right and left sides respectively. (Omitting minor though not inconsiderable sources such as the azygos and the coronary and pulmonary vessels) they come from the inferior and superior caval streams. The superior caval stream seems to go entirely to the right side and about one-quarter of the inferior caval stream also goes to the right side, the remaining three-quarters going to the left side. Thus, of the 3 c.c. which leaves the right side, 1 c.c. comes from the inferior caval stream and 2 c.c. from the superior caval stream, whilst of the 3 c.c. leaving the left side, all come from the inferior caval stream. If these figures be accepted, four c.c. of blood will reach the heart from the inferior vena cava for every two which reach the heart from the superior vena cava. Turning to the destination of the blood leaving the heart, we may start from the fact that the blood going to the head and arms is the same in quantity as the blood returning from the superior caval confluent and is therefore 2 c.c. for each 6 which leave the heart and 2 c.c. for each 3 which are expelled into the ascending aorta; the remaining 1 c.c. traverses the isthmus of the aorta, reaching the descending aorta, where it meets the stream (3 c.c. from the left side of the heart) which enters the dorsal aorta via the ductus arteriosus, making 4 c.c. in all.

We have already reason to believe that the placental circulation amounts to one-half the output of the heart and therefore accounts for three of every four cubic centimetres passing down the dorsal aorta. These 3 c.c., of course, pass in time to the umbilical vein and constitute three-quarters of the blood traversing the inferior vena cava.

XIV. NERVOUS SYSTEM

Knowledge of development of function in the nervous system of the mammalian embryo lags far behind that of structure. A complete picture is not made easier by the apparent differences which are observed between one species and another. Two obvious approaches exist to a study of the subject, the behavioural and the anatomical. The former consists in observing primarily the movements of the foetus and then endeavouring to relate them to the anatomical development, the latter observing primarily the anatomical development and endeavouring to relate it to the observed movements. These two methods have led to different theories. The present brief treatment at the outset discards theories and purports to summarise the principal known facts. Apart from movements which are under the suspicion of being purely muscular, the first movements which can be elicited from a mammalian foetus are reflex (for summary *see* Barron, 1941). The animals observed were the rat, rabbit, cat, guinea-pig, sheep and man. In every case but one, the first region, stimulation of which elicited a movement, was the face. One author (Coronois (1933), cat) brackets the paw with the face. The precise area on the face is not the same as noted by all observers. The point, however, is that the nerve involved is the fifth cranial nerve. With regard to the cranial sensory nerve below the fifth, we have no information. The response observed differs in detail according to different authors, most of whom have specialised in a particular species, but in nearly every case the response is some sort of movement of the neck muscles.

Because the sheep foetus develops slowly and is relatively large, it is a more satisfactory form on which to work than the smaller embryos of the rabbit. It furnishes also a fairly complete picture which can be used to constitute a base line for the comparison of results obtained from other animals. Let us confine ourselves for the present to the sheep. In considering the sheep, it must be borne in mind that a latitude of about a day must be allowed in the foetal ages as stated between the insemination of the ewe and the actual fertilisation of the ovum and so forth. Thus, if a foetal age were given as 30 days, the foetus might in reality be only 29 days old—clearly it could not be 31 days! The first intrinsic movements of which we have any record are on the 32nd day. These were obtained by direct faradisation of the muscles of the foreleg and are probably not neuromuscular movements, or if they are they are the result of direct stimulation of the nerve endings in the muscles. The point is they are purely of local importance.

On the 33rd and 34th days movements believed to be neuromuscular have been elicited in three ways; (a) by tapping the amniotic sac (the wall of the uterus having been dissected); (b) by manipulation of the uterus (the so-called spontaneous movement); (c) by stimulation of the face just underneath the eye with a glass rod. With regard to (a) and (b) it is not clear what the receptor path or paths are. With regard to (c) the receptor path seems to be the maxillary branch of the fifth cranial nerve, for the sensitive point is just above that to which the nerve has grown—presumably the tap on the skin stimulates the naked nerve endings just underneath.¹

¹ The conception that immature terminal points of nerves are sensitive to stimulation in the foetus (man) is supported by the work of Ira Dwight Hogg (1941). "It is suggested that early excitation of sensory nerves is probably dependent on deformation of the growing tips of fibers by displacement of the surrounding tissue."

The response in each case is a movement which may reasonably be referred to the neck muscles, either turning of the head, extending the neck, or a movement of the position of the arm. The case of (c) furnishes a definite reflex arc commencing with receptors in the maxillary branch of the fifth cranial nerve. The impulse would seem to pass thence to the reticular formation and thence by the reticulo spinal tract to efferent somatic fibres operating the neck muscles involved. The development of this reflex may be followed from day to day. Four alterations appear with time :

(1) The area, stimulation of which evokes a response, increases as the maxillary branch grows and as the ophthalmic branch becomes sensitive. (It is not possible to use the word sensitive in any strict sense ; the end of one nerve fibre may elicit a response to tactile stimulation when another does not, not because it is inherently more sensitive but because it is more accessible.) Thus, before the 39th day the snout and eye become specially sensitive.

(2) A smaller stimulus, if it is diffused, such as the falling of a drop of saline on the eye region, will produce a response.

(3) The response may consist in not a single movement but the movements may be repeated, producing a rhythm.

(4) As the foetus grows older, the movement becomes more and more extensive, starting on the 33rd-34th day with the neck, by the 36th day the back and the fore limbs are involved, but not the hind limbs ; by the 39th day the hind limbs are involved. The development of the above response to the point indicated, i.e. a rhythm of similar spasms, decreasing in violence as the response passes off, ends a definite stage in the development of the nervous system at about the 40th day.

Up to this point it may be claimed that whether the stimulus is a specific one to the nerve endings of the fifth cranial nerve, or whether it is a rap on the outer surface of the amnion, or a manipulation of the uterus, or even faradic stimulation of the medulla, the response is much the same and is as described above. The question naturally arises : Up to 40 days is the nervous system of the sheep capable of any other response ? So far in our experience, mechanical stimulation of no area served by nerves other than the fifth cranial nerve has given a response before the 40th day. On the 41st, flipping the foreleg produced flexion of itself and a movement of the crossed leg, head and tail.

Up to this stage probably nothing in the central nervous system above the medulla has been involved. The justification for this statement lies in the fact that if at a later stage, say 60 days, the brain be transected just below the pons, allowed to grow in the uterus (as it will do) and examined, say, ten days later, the only movements of which it is capable are of the same general character as those described.

After the 41st day, certain alterations in the type of movement appear :

(1) A distinction appears between the movements of the neck, trunk (apart from the muscles involved in respiration) and limbs, which assume a deliberate and sustained character, and those of the diaphragm and other respiratory muscles which retain their spasmodic and rhythmic character. A typical response consists of both the above types of movement. In the earlier days of the 40-49-day decade, the somatic spasm and the respiratory rhythm commence simultaneously but the latter outlasts the former. Towards the end of the decade

the somatic spasm definitely precedes the respiratory rhythm and by the 49th day there is a definite gap between the two.

(2) About the 45th day an asymmetry in the somatic movements commences to appear and shows itself in two ways :

(a) A gravitational response appears. If the foetus lying on its side moves its head, there is a torsional movement of the neck, the crown of the head being turned towards the sky. This seems to depend, not on the semicircular canals, but on the unequal strain imposed by the weight of the head upon the neck muscles.

(b) Movements of the limbs on torsion of the head similar to the " Magnus and de Klejn " movements.

(3) The area, stimulation of which produces a response, becomes extended practically to the whole body.

(4) The response to stimulation of a reflexogenous area seems to have three properties :

(a) With regard to the extreme spread of the response, this is at first localised. As the foetus gets older the field expands, but at a yet later date the field contracts and eventually becomes quite restricted.

(b) Within the framework of (a) the spread of the response depends upon the strength of the stimulus.

(c) If two stimuli are given with only a short interval between, the second will produce a less widespread response than the first.

(5) Generally speaking, the foetus becomes increasingly sensitive during this decade.

(6) The region of the pons is involved at this time, but not to any great extent the midbrain.

During the 6th phase, i.e. from 50-59th-day decade, the responsiveness of the foetus is waning. The foetus is at its most sensitive phase about the 50th day at which time it is in almost incessant movement. It is difficult to hold the embryo so still as not to initiate rhythms of respiratory movement. By the 60th day the foetus is extremely inert. It behaves as if drugged ; indeed, the impression given is that of an embryo which has fallen on evil days and for some unassignable cause lost its vitality. That this is not so can be shown in two ways :

(1) After a certain amount of exposure the foetus wakes up and becomes quite lively.

(2) If an extra-uterine transection of the brain be made just below the mid-brain in one of two twin embryos, that embryo on subsequent examination will be extremely active, whilst the twin, the brain of which has had no lesion, will be extremely " sleepy," only itself to wake up and become lively after considerable exposure, or occlusion of the umbilical cord. Evidently some sort of inhibition is exerted on the mobility of the foetus from a centre or centres situated above the pons. At this age if the connection between the region posterior corpora quadrigemina and the parts below it are intact, it seems to make little difference whether that region is cut off from the parts of the brain above it or not.

The onset of inhibition has been observed in man by Hooker : " By about 16 weeks of menstrual age, an interesting change in the activity of the foetus has appeared. The younger foetuses observed have been active in their response to

stimulation, but they now show a marked decrease in activity, becoming almost sluggish, although a few individuals have been observed which were still fairly active in their responses. Furthermore, where it may be accomplished, resuscitation causes disappearance of this inactivity and increase in threshold, otherwise rather characteristic of the period from 16 weeks to birth. This phenomenon is not limited to man, as Barcroft and Barron have noted in sheep embryos."

Between the 50th and 60th days another important change is taking place, indeed signs of it are evident even before the 50th day. Rhythmic movements of the diaphragm occur unaccompanied by movements of the somatic muscles (apart from the muscles of respiration). Thus we have the establishment of a definite and separate function as such, so far as muscular movement is concerned. At a later stage a further "dose" of inhibition seems to be imposed from a centre in the diencephalon, or perhaps the locus is shifted in some degree from the P.C.Q. to the upper part of the diencephalon. Decortication of the brain in sheep foetuses does not seem to affect the cortical movements.

After the 60th day respiratory movements do not seem to take place without some definite stimulus.

By about the 70th day (i.e. half-way through the gestation period) everything essential seems to have been laid down in the sheep; the second half of the gestation period is spent on the development of what is already in being.

From the above description, certain points in the functional development of the nervous system emerge:

(1) The first part of the brain to exercise a receptor function is the lower part of the medulla. This is evident with regard to the descending nucleus of the fifth nerve; about the tenth we have no information, as the sensory nerve endings are inaccessible.

(2) From this point the C.N.S. develops functionally progressively forwards and progressively backwards, the upper part of the medulla, the pons, the mid-brain and the forebrain, commencing to function in that order.

(3) In a general sense the spinal cord develops functionally from above downwards. This appears to be subject to another consideration, namely, that the segments containing a large number of nerve-cells appear capable of exhibiting reflexes relatively earlier than the segments containing few nerve-cells. "Appears," because the appearance may be misleading. It may be that if you have two reflexogenous areas, one contributing X-cells and the other Y-cells, and if Y is much greater than X, an effect may appear earlier from stimulation of the Y-cell area, though cell for cell there be no difference, the cause being the cumulative effect of the number of receptors stimulated.

The central nervous system in the human foetus.

The principal points of interest in the study of the human embryo are:

(1) That being of much slower development even than that of the sheep, the stages of development are more "strung out";

(2) The final phases lead up to movements if we are objectively conscious and can describe and explain in terms of our own experience.

The worker is therefore inclined to divide the life of the human embryo into three periods:

(a) That in which there is yet no movement,

(b) That in which reflex movements can be evoked but in which they have not reached the stage of combining to form complicated and purposeful patterns, and

(c) That in which reflexes, such as the grasp reflex, are in essence what occur within human experience.

The difficulties of work on human foetuses are self-evident ; they boil down to the following : Whereas in the study of a sheep foetus the ewe is sacrificed to the condition of the foetus, in the case of the caesarian section the fate of the foetus is of no account to the surgeon—that of the mother is all-important. Clearly it would be unthinkable to prejudice the immediate health of the mother, or her efficiency as a producer of further children, for the sake of obtaining a revealing film of a foetus that was too young to live.

For a description of the development of reflexes as observed in human foetuses just detached from their mothers, I must refer the reader to a small book :—*The Origin of Overt Behaviour*, by Davenport Hooker of Pittsburgh. In this book the reflexes are tabulated as described in detail ; it contains so much information (the work of a great part of a lifetime) condensed into so small space, that I can condense it no further. Nor does the present author feel that he would have added anything of value to Dr. Hooker's observations but for the enterprise of his colleagues, Dr. I. M. Titcomb and Dr. M. M. Deane (1946), of West Middlesex County Hospital, who have given him the opportunity of studying the foetus *in situ* in the sac, and thus observing it in an earlier stage than did Dr. Hooker, as well as in its normal fluid environment. For this purpose, only mothers who, for some medical reasons, have been condemned to have no further children can be used. The collection of such material must in any case be slow, and to obtain it over the complete gestation period, even slower, because the operation would not take place unless the medical opinion deemed that the woman would not stand the strain of pregnancy. Thus it usually does not take place until pregnancy becomes a strain, and as soon as that occurs, the foetus is removed. Only occasional cases have come within the author's experience except between the 90th and 130th days. What seems possible is to see whether the available cases can be "spotted in on the background of the sheep," always remembering that the human foetus leads up to a biped animal with massive legs and clever hands, the ovine foetus to a quadruped.

The first reflex movement, as observed by Hooker and by Fitzgerald and Windle, is substantially the same as that in the sheep—stimulation of terminals of the maxillary branch of the fifth cranial nerve causes a movement of the head, but of the head only. This is at about $5\frac{1}{2}$ weeks. (This I have not seen but I fully accept it.) The only movement I have seen at 57 days is a lifting of the arm due to a tap on the top of the shoulder. It is doubtful whether this movement is a reflex. It may well be a direct stimulation of the muscle such as Hooker describes. Thus the human foetus at about 55 days corresponds to the sheep foetus at 34 days. At 72 days the human foetus when first seen in the sac exhibits rhythmic movements on displacement of the sac. These involve both the arms and legs more obviously than other parts, but if the foetus is on its back, they have been noticed to involve the trunk muscle, and the descent of the liver, presumably due to the involvement of the diaphragm. The body movements may be seen immediately after puncturing the sac if the foetus is placed on its back with the

umbilical circulation still intact. I have not seen it in a foetus removed from the mother ; in this my experience verifies that of Hooker. At this point the 75-day human foetus corresponds to the 40-day sheep foetus.

" Spontaneous " rhythms of this general type were observed on the 80th day.

On the 95th day, the " spontaneous " movements observed when the foetus was first seen in the sac had altered in the sense of an accentuation of the leg movements and fading of the arm movements. The foetus when first seen was kicking ; the arms moved, however, when the arms were touched. When the foetus was delivered on its back, rhythmic movements as the result of no specific stimulus, involving chest and lower part of body, including flexion of legs.

On the 102nd day, " spontaneous " movements seen on first observation of sac were rhythmic movements of the arms and legs, the arm movement included a fanning-out of the fingers. The rhythm soon ceased : when the sac was opened and the foetus placed on its back the arm movements did not occur. The rhythm involved the muscles of the chest and abdomen and the leg ; the last persisted longest. Local reflexes good. Turning head produces limb movements of no very specified pattern. This suggests the first indication of the Magnus and de Kleijn reactions, and therefore a correspondence with about the 45th day in the sheep, but nothing has been seen quite like the complex consisting of the " somatic leap followed by the respiratory rhythm " so evident in the sheep at 45 days.

Some specific reflexes.

We have now reached the 14th week, by which time, in the words of Ira Dwight Hogg, "the type of response changes from simple avoiding movements to nearly all the recognised specific somatic reflexes." Whether or not the simple movements of the earlier weeks are avoiding movements is a matter of personal judgment and probably would not be admitted by Windle, but the specificity of the later responses is not in doubt since they persist into adult life, and therefore we have our own experience to vouch for these. Some of the more prominent may be discussed. The plantar reflex has been very completely studied by a number of observers, of whom the pioneer was Minkowski. Hooker gives the following dates : at 77 days (approx.) as the date of the first appearance of a plantar reflex, at which age the toes are turned downwards towards the sole (plantar-flexion) ; at $11\frac{1}{2}$ weeks the response to stroking the sole may be either plantar-flexion or dorsiflexion (the extension towards the dorsum of the foot), of the toes without toe-fanning. By the twelfth week the foot sole response is progressing towards completion, the toes plantarflexing or dorsiflexing with some fanning. Minkowski lays great stress on the development of the plantar reflex because he regards this response (plantarflexion or dorsiflexion) as an index of the level in the central nervous system at which the receptor stimulus is turned into an effector one. He visualises the reflex as follows :

Length 3-5 cm.	2nd month. Early embryonic neuro-muscular reflexes.
Length 5-15 cm.	3rd to 5th month. Early spinal phase.
Length 16-30 cm.	4th to 6th month. Middle embryonic tegmento spinal phase.
Length 31 cm.	7th month onwards. Late foetal pallido-rubro-cerebello-tegmento-spinal phase.

Of the reflexes in course of development at about 11 weeks, that which makes the greatest aesthetic appeal is perhaps the grasp reflex. The elaboration of the grasp also furnishes a good example of just how such reflexes are developed. It has been studied in detail by Davenport Hooker (1944). To quote him :

" If the palm of the hand is stimulated with a relatively pliable hair at this age (11 weeks) a quick closure ensues. A study of the motion picture proves the finger closure to be only partial. Halverson (1937) has divided prehension into two phases, finger closure and grasp. These two phases are sequential in development. No grasping is present at this stage, indeed, as I have indicated, the initial finger closure exhibited at 11 weeks is incomplete. In this finger flexion, the thumb may or may not be involved. In earlier observations at this age, thumb movement was absent, but it has been seen to occur in more recently observed specimens of this age. . . . At 12 weeks digital closure is still only partial. At 13 weeks thumb opposition though only partial has been observed when the other digits flexed. Finger closure may be complete at 13½ weeks. At 15 weeks if a hair be drawn across the palm, the fingers not only close but remain closed, particularly if the hair is left within the closed fingers.

" This seems to be the initial appearance of the grasping reflex though there is no effective clutching at the object within the palm."

It has been our experience that at 17 weeks the arm may be extended by placing a probe in the hand and gently raising it. Finally, at 22 weeks, according to Hooker :

" The ability of the fetus to grasp an object has reached a point in the maturation of this reflex at which it is somewhat effective. It is sufficiently strong to enable the observer to move the upper extremity by pulling a grasped rod in the direction of its long axis."

Such, then, is an account of the development of a single reflex, and having given a picture of it as well as of the general development of the nervous system in its earlier phases, we must end this review of the physiology of pre-natal life.

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CHAPTER 18

CHANGES IN THE MATERNAL ORGANISM DURING PREGNANCY

By W. H. NEWTON

The subject of this chapter embraces the whole physiology of the mother, and the discoveries bearing upon it have been made by specialists in many fields. The writer cannot hope to do equal justice to them, and although references to books and journals outline the foundation on which the text is laid, they are no more than a series of clues to the vast literature which exists. A review or paper with a good historical bibliography is frequently cited in place of the historical contributions themselves, a practice open to criticism but not, it is hoped, indulged in to excess. The majority of investigations have been made upon women, often for clinical rather than scientific ends, revealing innumerable facts of interest, but findings which do not, at present, seem relevant to the systematic scientific understanding of the subject are here neglected. Space will also be saved by our making no attempt to chronicle all those who have passed along the well-trodden paths, for among the latest and technically best-equipped travellers one will usually be found who has made the necessary recapitulation.

Behind nearly all clinical appraisals of the normal pregnant woman has lain the shadow, so far impenetrable, of the toxæmias of pregnancy. The question asked has been not "What is pregnancy?" but "What is normal?" in the hope that the knowledge sought will enable the abnormal to be controlled. The facts are as far as possible presented so as to reverse this bias. Similarly, the temptation to discuss nutritional deficiency in pregnancy has been resisted. Although fundamental research has its bearing on these pressing and important problems, they are themselves the concern of the clinician, the dietitian and the sociologist.

Some of the points at issue may be stated in a few words.

In many animals the weight of the litter is an appreciable proportion of that of the mother. For instance, it is common for a mouse to have a litter of 10 young, each weighing nearly 1 g. at birth. It would be incredible that foetuses attaining such a weight had lived on the maternal tissues as parasites, and it is reasonable to suppose that the physiological economy of the mother is so arranged during pregnancy as to allow for the growth of the embryos without obligatory sacrifice on her part. In other animals, such as man, in whom the foetus weighs but little in comparison with the mother, superficial observation would not lead to the same conclusion. An important question we have to consider is whether, in the light of experimental evidence, the mother is actually better or worse off at the end of pregnancy than she was at the beginning.

We shall see that under normal circumstances she may be materially better off, thereby raising the question of the precise use to which the additional food

assimilated during pregnancy is put. Some of it is transformed into foetal tissue, but it is evident that this, as its amount increases, must require for its own life processes more and more fuel. The most incalculable of these processes are growth and development themselves, which may entail a greater expenditure of energy than does the mere maintenance of life. Experiments on energy exchanges have yielded much information, but no detailed understanding of the purposes for which the energy is used.

The supply of raw material, whether for building the bodies of the foetuses or for fuel, introduces the problem of transport. Not only must the materials themselves be carried, but also the oxygen which enables them to be used and the waste products, including carbon dioxide, which result from foetal metabolism. While the immediate exchange of the various substances between mother and foetus is discussed in Chapters 16 and 17, we must look for modifications in the maternal circulation and respiration.

The metabolic, circulatory and respiratory adjustments of the mother cover many of the facts with which we must deal, but in using them as a basis for discussion we shall find that no system of the body can be neglected. Before embarking upon the details of the various adaptations, for each of which there often seems to be a separate "explanation," it will repay us to survey certain general features of the pregnant state.

I. THE STIMULUS FOR THE MATERNAL CHANGES

The view has gained ground that the satisfactory and economical relationship between mother and foetus, which must be the basis of pregnancy, is brought about by a change in the physiological organisation of the mother, whereby the wants of the foetus are largely anticipated. Although this supersedes the idea that the mother sustains a continuous hand-to-mouth response to the demands of the foetus, a stereotyped pre-adjustment in animals which produce a number of young simultaneously could not cater equally efficiently for small and large litters. We expect to find, therefore, either that the adaptation is sometimes imperfect, or that its magnitude is related to the size of the litter. In either event we need to examine the case for the state of the mother at any stage of pregnancy being predetermined.

If the condition and functional activities of the mother at any moment were solely the consequence of the wants of the foetuses at that moment, then, on surveying the *manmalia* in general, we should expect to find that pregnancy would terminate at some common stage of foetal development or of size relative to that of the mother. Yet, if we take three animals in ascending order of size: mice, guinea-pigs and rabbits, we find that pregnancy is longer (63 days) in the guinea-pig than in either the mouse (19 days) or the rabbit (31 days), and that the difference is a real one, because the newly-born guinea-pig is a much more highly developed animal than either of the other two. It is to be presumed that in the course of evolution the duration of pregnancy in an animal has become established in the same way as its other characteristics, and that it is appropriate to the habits and survival of the species. The immediate interests of both the foetuses and the mother are clearly reconcilable with widely different spans of gestation and conditions of foetal size and development at parturition.

There is room for much speculation and discussion about points such as these, and the difficulty of assessing all the factors involved is fully recognised. It will be sufficient for our present purpose if it is accepted that there is a *prima facie* case for regarding pregnancy as comprising the mutual relations of three participants—the foetus, the mother and the mechanism which organises the pre-determined characteristics of pregnancy in the animal concerned. We have used the gestation period as an example of such a characteristic. It may be argued that the components of this mechanism must be either maternal or foetal; nevertheless, it is convenient to keep it in a separate category and avoid trying to define its scope too exactly. It is frequently taken for granted. For example, Lochhead and Cramer (1922) start from the assumption that “the phenomena of pregnancy and parturition are brought about by chemical stimuli acting through the blood stream,” and they go on to quote the then available evidence on the function of the corpus luteum, and enter upon much speculation relating to the placenta as a possible source of the stimulus.

Today, a certain amount of direct evidence is available that the placenta holds a key position in determining the framework within which gestation occurs. Klein (1933, 1935) removed the foetuses from a 15-day pregnant rabbit, and, leaving the placentae in position, found 5 days later that the endometrium and myometrium were still typical of pregnancy. As a result of similar experiments, in which, in addition, the effect of presence and absence of the ovaries was tested, he postulated a trophic effect of the placenta on the corpus luteum. The placentae of the rabbit are not retained very consistently under these conditions (Hammond, 1917), and it fell to Selye, Collip and Thomson (1935) and the present writer (Newton, 1935) to show that, in rats and mice respectively, placentae without foetuses were retained and delivered at full term. In this and subsequent researches (Brooksby and Newton, 1938; Newton and Lits, 1938; Newton and Beck, 1939; Deanesly and Newton, 1941; Newton and Richardson, 1941; Gardner and Allen, 1942; Hall and Newton, 1946) it was found that, not only were the placentae delivered at the proper time for parturition (which involved a maternal loss of weight greatly in excess of the placental weight) but that while they were *in utero* oestrus was suppressed, the body weight already gained was maintained, the mammary glands developed normally, and the symphysis pubis separated naturally with the formation of an interpubic ligament. In other words, the animal was pregnant in every respect except for the absence of that part of the embryo which represented the future individual. With regard to other components of the reproductive mechanism, the ovaries were found to be implicated in two ways; first, the placenta had a definite trophic effect on the corpora lutea, and in the absence of the ovaries abortion occurred. Second, the symphysis pubis did not separate in the absence of the ovaries when pregnancy was maintained by chance or as a result of administering progesterone. The pituitary gland was found to be unnecessary for mammary development, pubic separation, maintenance of the corpora lutea and parturition, but in its absence lactation did not occur. The main original findings were early confirmed by Kirsch (1938) for the rat, and Astwood and Greep (1938) prepared luteotrophic extracts from rat placenta.

Van Wagenen and Newton (1940, 1943), Dorfman and van Wagenen (1941) and van Wagenen and Jenkins (1943) have shown that after removal of the foetus

in *Macaca mulatta*, the placenta being left *in situ*, the animal remains physiologically pregnant by the following criteria until parturition occurs at normal full term (13 to 84 days after the operation): (1) after a preliminary pause following the operation, and if there is then time before parturition, the gain in weight of the animal is resumed (Fig. 18. 1), (2) the intensification of colour in the sexual skin, the generalised oedema, and the characteristic inactivity of pregnancy persist (3) the hydro-ureter of late pregnancy persists (it may even appear), but disappears with the delivery of the placenta. (4) the urinary concentrations of oestrogen and androgen remain typical of pregnancy. In the monkey, the ovaries are not necessary for the maintenance of placental pregnancy.

These experiments, together with various scattered observations by other investigators on the effects of retained placentae, are sufficient to prove that the foetus and the state of pregnancy are not inseparable. The exact nature of the pregnancy-maintaining mechanism remains to be determined, but it is evidently dependent on some or all of the hormones secreted by the placenta (Chapter 16) which seems capable of superseding to a varying degree both ovaries and pituitary.

In a recent important paper, Hisaw (1944) postulates for the monkey a chain of hormone combinations leading up to the complete dominance of the placenta. He calculates that the ovum becomes embedded before the death of the corpus luteum of menstruation, and supposes that the chorionic gonadotrophin which it introduces into the maternal blood stream prolongs the life of the corpus luteum for a further 15 or 16 days. The latter estimate is based on the demonstration of a limited luteotrophic action of human chorionic gonadotrophin on monkey corpora lutea and the total unresponsiveness of these to pituitary luteotrophin at this stage (Deanesly and Newton, 1941, found the luteotrophic action of the mouse placenta to be independent of the pituitary). The end of this period coincides closely with the time during pregnancy at which the monkey's own chorionic gonadotrophin can be detected in the urine, and Hisaw points out that "Chorionic gonadotrophin in the blood and urine of pregnant women, chimpanzees, and monkeys attains its greatest concentration in early pregnancy at a time when the placenta is undergoing its most rapid development and is becoming established in the endometrium. In fact, in the chimpanzee and monkey it has been detected only during this period." Hisaw further raises the possibility that during the prolongation of its life the corpus luteum secretes oestrogen, and continues to do so after ceasing to produce progesterone. The ovaries become

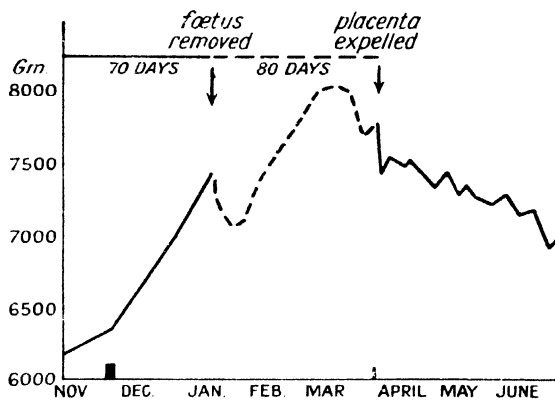


Fig. 18. 1—The weight of a monkey throughout pregnancy and for 3 months after parturition. The thick datum mark covers the days on which the fertile ovulation could have occurred. The thin datum mark signifies parturition. The broken line indicates the period after removal of the foetus when only the placenta remained in the uterus. (From van Wageningen and Newton, 1943.)

unnecessary for pregnancy at about this time, but it is by no means improbable that in the normal course of events they contribute oestrogen for some time longer, for the oestrogen found in the urine in human pregnancy after oöphorectomy is not impressive in amount, and may at first disappear (*see* Newton, 1938).

It will be noticed that while attention has naturally been focused on the organs which are more obviously concerned in pregnancy, there are indications (e.g., the weight changes) that the mother is extensively affected. Whether the metabolic and other effects of pregnancy we shall describe will be traced to the readjustment of the endocrine balance, for which the placenta seems so largely responsible, cannot be predicted. It can no longer be taken for granted that they are the result, literally, of the "demands" of the foetus. Nevertheless, it would be a mistake to imagine that the foetus is altogether a passenger. Even in the mouse, where placental pregnancy so closely simulates the normal, the act of parturition may be very sluggish, and in the monkey with a retained placenta mammary development is almost certainly subnormal. In the rabbit, placental pregnancy is either ill-maintained or incomplete. Wishart and Hammond (1933) have shown in rabbits that the greater the number of foetuses, the less each one of them weighs at birth. Moreover, large litters may be born as much as four days earlier than small litters (Table I). While this may be due to a quantitative difference in the endocrine stimulus, the total weight and bulk of the litter cannot be excluded as the determining factors. Reynolds (1939) has treated the influence of uterine distension in some detail, and has gone to some trouble to plead for a recognition of the multiplicity of factors concerned in pregnancy.

II. ENERGY EXCHANGES

Leaving the question of the causation of the maternal changes, and entering upon a description of them, it is best to deal first with the question which comes next in order of generality—that of the overall increase in energy production by the mother.

It may be convenient to the reader to be reminded at this point that for whatever purpose food material (stored or ingested) is oxidised in the animal body, the energy so produced is degraded to heat. By means of elaborate apparatus, the total turnover of energy can be assessed by measuring this heat production directly. It is, however, more usual to measure the rates of oxygen consumption, carbon dioxide production and nitrogen excretion, and from them to compute the weights of fat, carbohydrate and protein oxidised during a certain time. A further simple calculation based on the known calorific values of these types of food gives the total heat production to which their breakdown is equivalent. The rate of heat production of an animal or person is usually expressed as Calories per kilogramme of body weight per hour, or Calories per square metre of body surface per hour, or sometimes merely as Calories (per individual) per day. It is known as the *metabolic rate* (MR) at the time of the experiment.

The greater the amount of muscular activity, the greater is the MR and it varies from moment to moment during the day, unless the subject maintains an absolutely steady state of activity or absence of activity. The latter is easier to achieve, and under equable and constant conditions of temperature, and 12 to 18 hours after a meal, the resting MR is constant for the individual and is called the *basal metabolic rate* (BMR).

In individuals of the same build, the BMR per unit of body weight is constant for the species, and normal standards for human beings of all shapes and sizes have been established. The BMR for smaller animals is greater per unit of body weight, and in the mouse,

TABLE I
(Wishart and Hammond, 1933)
MEAN BIRTH WEIGHTS IN GRAMS (RABBITS)

Duration of pregnancy (days).	Number in litter.												Weight mean.
	1	2	3	4	5	6	7	8	9	10	11	12	13
31	—	—	—	39.0	46.8	43.8	49.2	54.7	51.1	51.0	47.0	42.8	45.8
32	—	76.0	58.0	68.6	52.5	53.5	58.1	53.3	55.5	49.1	41.9	—	43.0
33	103.5	77.5	70.4	66.6	54.8	62.1	52.8	40.6	49.2	59.5	—	—	60.
34	92.0	84.6	72.7	72.2	—	—	57.9	66.9	—	—	—	—	68.6
35	91.0	—	—	—	—	—	—	—	—	—	—	—	91.0
Weighted mean	97.5	79.7	69.5	66.0	53.0	57.3	53.7	53.7	53.1	51.4	45.5	42.8	54.3

for example, is 15–20 times greater than in man (Chevallard, 1935 ; Barbour and Trace, 1937). If a series of animals of different sizes is taken, the BMR is found to be proportional to the 0.73 power of the body weight. The surface area of the animals is proportional to the 0.66 power of the body weight, so the BMR per square metre of body surface is, to a rough approximation, constant, a fact which was early discovered.

The increase in total metabolic rate.

All observers are agreed that the BMR increases during pregnancy, not only for the individual as a whole, but per kilogramme of body weight. The earlier

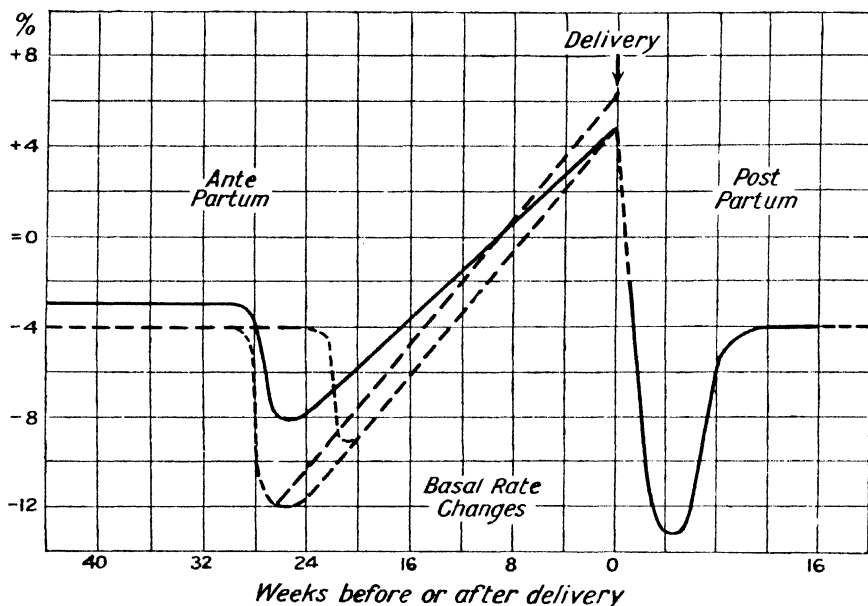


Fig. 18. 2—Variations of observed from predicted BMR during pregnancy in women. The unbroken line gives the average of 77 cases studied by Rowe and his associates. The broken lines represent the cases of Boothby, Sandiford, Root and Root respectively. (From Rowe, 1931.)

experiments on oxygen consumption by Magnus-Levy (1904) and Zuntz were regarded by Murlin as inconclusive, but later experiments by Zuntz (1910) and Carpenter and Murlin (1911) indicated that the metabolism of women increased towards the end of pregnancy. (The experiments of Carpenter and Murlin were interesting because the heat-production was measured directly with a calorimeter. Unfortunately, 8 different women were used to provide a figure for the "normal" BMR.) Since then, Root and Root (1923), Rowe, Alcott and Mortimer (1925), Sandiford and Wheeler (1924), and Johnston, Hunscher, Hummel, Bates, Bonner and Macy (1938) have recorded painstaking studies of single cases whose BMR's have been determined at frequent and regular intervals during pregnancy and lactation. (Bokelmann and Scheringer, 1931, and Jensen, 1938, give more complete lists of earlier researches.) Rowe and Boyd (1932) have presented a series of observations on no fewer than 77 women extending over the last 6 lunar months of pregnancy, and their results are epitomised in Fig. 18. 2.

The dips of the average curve below the resting value which occur between the 12th and 24th weeks of pregnancy and during the first 8 weeks of the puerperium are unexplained. The fact that they are paralleled in the findings of the two other investigations which Rowe and Boyd superimpose on their own graph is interesting.

The results of inquiries in domestic and experimental animals are reported by Brody, Riggs, Kaufman and Herring (1938) and Brody (1938), who also quote previous work. They assume that if the BMR during pregnancy increases at a greater rate than does the 0.73 power of body weight, then it is abnormally high. By this criterion they find the BMR to be raised in cattle, horses, sheep, swine, goats and rabbits, but not in rats, during pregnancy.

The most important question which remains unanswered about the increase of BMR during pregnancy is: precisely to what is it attributable? Since the overall energy production per kilogramme is increased, it follows that the foetus cannot be regarded as a mere addition to the size of the mother; the tissues of either foetus or mother or both are transforming energy at an abnormally high rate. If the mother, we should like to know whether the rise is due to hypertrophy of the uterus, mammary glands and other organs upon which pregnancy throws an unusual burden, or whether the increased BMR is a by-product of the endocrine readjustment of pregnancy—a consequence, for example, of increased thyroid activity.

These questions have not yet been unequivocally answered because, although it is easy to follow the MR of the pregnant mother, no satisfactory means of making more than an estimate of the foetal contribution have yet been devised. The evidence and opinions which bear on this point are, however, of some interest.

The eggs of marine organisms, insects, and especially hens, provide an opportunity which was seized very early of measuring the MR of embryos. Rubner (1908) and Murlin (1910) quote the results of such experiments, particularly those by Tangl and Bohr, and they also refer to the well-known work of the latter on guinea-pigs, whose metabolic rate he measured before, during and after clamping the umbilical vessels of the foetuses. From a survey of this evidence, Murlin is able to conclude that "the energy metabolism of the embryo is greater per unit weight than that of the mother." From the later review by Needham (1931), who draws upon a larger population of experiments, the further conclusion emerges that not only is the MR of the growing organism not constant, a fact which was obvious from the work of the earliest observers, but it may rise from a low level in the fertilised egg to a peak at some later stage, and then decline towards the adult level. The peak may occur during or after embryonic life, in the later phases of which the MR may therefore be rising or falling. This generalisation may be premature, but it is almost certainly true, because, as various workers have pointed out, if the MR per unit weight is followed backwards from adult life, the rise found during the time of growth and development would, if continued right back to the moment of fertilisation, presuppose an extremely high MR at that time.

Is the purely maternal metabolism raised?

The fact that the embryo for part, if not the whole, of its dependent existence has a higher MR per unit weight than that of the adult does not help us to decide

whether, or how much, the maternal metabolism is also raised, because the available data are not sufficiently complete. Information from the respiration of tissue slices strongly supports the view that developing tissues have a higher metabolic rate than adult tissue, but Anderson, Prest and Victor (1937) find that the metabolism of maternal rat tissues sometimes increases (e.g. the liver) and sometimes decreases (e.g. the kidney) in pregnancy. Such comparisons as have been made between adult tissues of different species reveal no relationship between the rate of respiration of the slices and the MR of the whole animal per unit weight.

In the absence of suitable determination of foetal MR some workers have resorted to the somewhat questionable practice of calculating it, and then subtracting the theoretical figure so obtained from the total MR of the mother. The method of calculation is not sufficiently reliable to be universally accepted, but it is important to understand something of what lies behind it, because the results of unquestionably good experiments may at first sight appear to support it.

We have referred (p. 446) to the empirically determined fact that the BMR of animals of various sizes is proportional to a certain power of their body weight, and very nearly to their surface area. The law holds good, at any rate approximately, for animals of the same species but of different size. Can it be assumed to hold good for the foetus? Rubner argued strongly, and most reasonably, that it could, if for no other reason than the unlikelihood of a sudden change in the MR of the foetus at birth. If it obeyed the law from the moment of birth, surely its MR must have been in the same neighbourhood just previously, otherwise the transition from intra-uterine to extra-uterine life could not be a smooth one. He recognised the effect of exposure and exercise on the MR of the young and concluded that the MR of the foetus just before birth (per unit weight) would lie about half way between that of the mother and the new-born animal.

It is unnecessary for us to go into the question of whether there is or is not a *causal* relationship between surface area and metabolic rate, or whether each is independently related to a third and more fundamental, if elusive, entity called "active protoplasmic mass." By reading Needham (1931) and Du Bois (1936), but not one without the other, the reader may place himself in a position to attack this problem. Meanwhile, to those who have hitherto accepted the "surface area law" too literally, and who may be disposed to argue that the foetus can have no effective surface, and need not therefore obey the law, it must be pointed out that any *immediate* dependence of the BMR on the surface and its function at a particular moment has long since been abandoned. The metabolic habit of the species is supposed to have been developed in the course of evolution, and a correspondence between BMR and surface area to have been favourable for survival. Rubner's assumption that the foetus passes smoothly from intra- to extra-uterine life is independent of any particular view of foetal metabolism.

An experiment by Murlin (1910) on a pregnant bitch showed that the excess metabolism 3 days before parturition over that during sexual rest was proportional to the weights of the litters subsequently delivered (two pregnancies were observed, one in which a single puppy and one in which 5 puppies were born). More striking was the fact that the excess metabolism in both pregnancies at this time agreed precisely with that calculated from the (supposed) surface areas of the foetuses. Pommerenke, Haney and Meek (1930) obtained similar results in rabbits. Murlin, however, regarded the excess MR of the pregnant animal as

partly maternal in origin, and an examination of his results shows that the MR 3 days before the first parturition was the lowest recorded in that animal for 16 weeks. Later, Carpenter and Murlin (1911) made similar observations on a woman in the course of which they were able to determine the MR of mother and baby together when both were quiet. The pre-partum and post-partum values were so similar that there was no break in the smooth increase of MR. It was taken for granted that the MR of the baby had increased as a result of its new environment, and that that of the mother had decreased as a result of involutionary changes. In other words, an element of coincidence in both canine and human experiments is really implied. Sandiford and Wheeler (1924), however, finding that the excess metabolism in their own human case, and in others at that time recorded in the literature, agreed with that calculated from the supposed surface area of the foetus at various ages, concluded that the energy production per unit weight of maternal tissue was unchanged during pregnancy.

Rowe and Boyd (1932) from their long series of cases, came to the opposite conclusion, namely, that there is an increase in the purely maternal heat production "apparently the result of a complicated and unknown mechanism, engendered by the state of pregnancy but involving other factors than that of foetal growth alone." With regard to the contention of Sandiford and Wheeler they say, after analysing the data at their disposal, that "the constancy of heat output in terms of surface area when the foetal area is added to that of the mother is apparently artificial and results from the application of a small progressive factor as the correction to a larger quantity also showing progressive incremental change, but at a slower rate."

The conclusive experiment has yet to be made, but the curious dips already referred to in the composite curve of Rowe and Boyd make it extremely doubtful whether the human foetus *per se* can be held responsible for the whole of the increase in the BMR of pregnancy, though it does seem probable that the foetal MR is higher per unit weight than that of maternal tissue. At birth the MR per unit weight of the baby approaches twice that of the mother. Although the infant possesses this high MR at birth, the peak of its metabolism per kg. is not reached until the age of 4 years. It does not, therefore, during the first period of extra-uterine life obey the surface area law, and at about 4 years of age its MR per sq. m. of surface is also at a maximum. If we work back from this peak, we find that the MR per sq. m. is steadily diminishing down to the time of birth, and there seems no reason to suppose that if we could follow it into intra-uterine life it would do otherwise than continue this trend. Observations on premature infants support this view.

Brody (1938) calculates for the animals already mentioned, as well as for women, what the foetal MR per kg. and per sq. m. would be, if the excess metabolism of the pregnant mother were entirely foetal in origin. He concludes that in all cases the foetal metabolism comes out impossibly high, and it is true (with one exception) that the values are between 2000 and 4000 Cals. per sq. m. per day for a large part of pregnancy. (The "standard" BMR for animals of all sizes is about 1000 Cals. per sq. m. per day.) The exception is the human being, in whom the discrepancy is not so glaring—it is not surprising, therefore, that a diametrically opposite conclusion could be drawn from the same data by Sandiford and Wheeler. We have, however, already dealt with pregnant women,

and notwithstanding the findings of Brody, Riggs, Kaufman and Herring (1938) on the rat, and of Murlin on the dog, it seems that the bulk of data support the view of an increase in the purely maternal MR.

Total Cals. &
Cals. per Kg.
1000 000

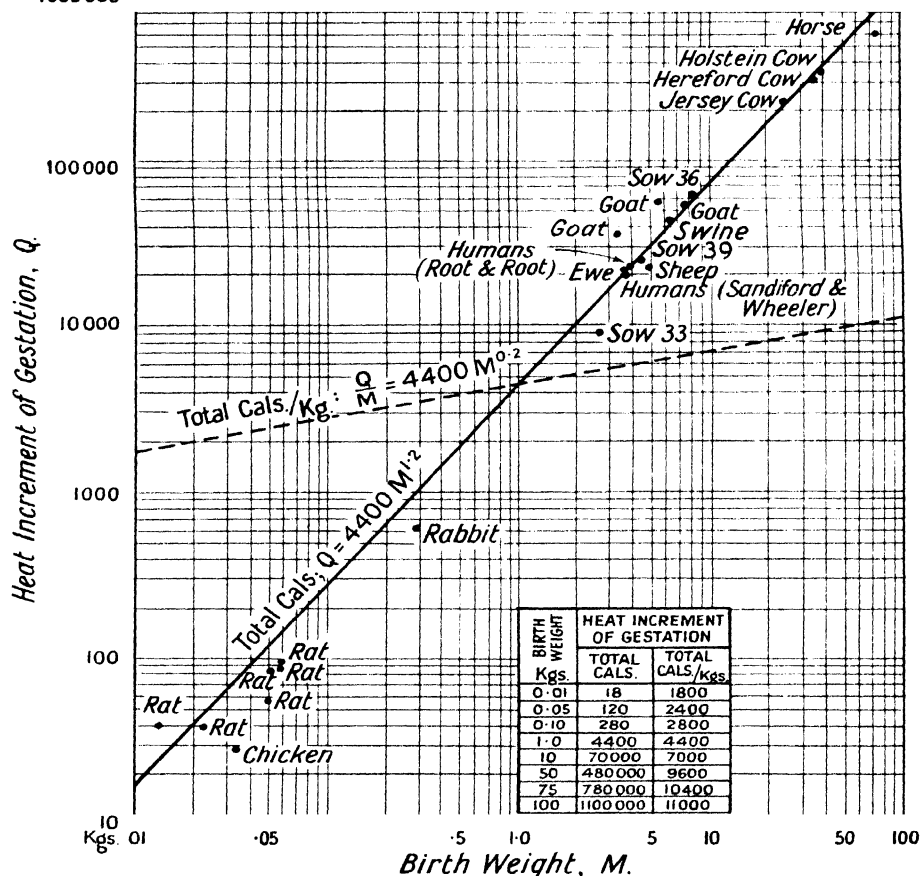


Fig. 18. 3.—The relation between the heat increment of gestation (Q) and birth weight (M) of the offspring plotted on a logarithmic grid. Against the birth weight are plotted (a) the ratio of the heat increments of gestation to birth weight (broken line), and (b) the total heat increment of gestation. (From Brody, 1938.)

The total excess metabolism of the pregnant animal, over and above what it would have been in the absence of pregnancy, appears to be related to the birth weight of the foetuses. Brody expresses the relationship between the total metabolic increment of gestation (Q) and the weight of the new born (M) as

$$Q = 4400 M^{1.2}$$

This is the equation to the continuous line of Fig. 18. 3, which also shows the experimental points to which the line is fitted. There must clearly be some question

as to the "correctness" of the slope (defined by the index 1.2) and of the constant 4400 (defined by the intercept of the graph with the vertical axis). But, that a relationship exists can scarcely be doubted.

The formula would mean that if the offspring at birth weighed 1 kg., the total cost of their production (excluding the potential energy represented by their own substance) would have been 4400 Calories. If the offspring weighed 10 kg., the total cost would have been 70,000 Cals. or 7000 Cals. per kg. of foetus; the cost for several weights of foetus is inset in Fig. 18. 3.

Whatever may be the relationship of the MR of the developing foetus of a single species to the surface area law, it might be supposed that the MR's of different species would bear the same relationship to each other in the foetal as in the adult state. A foetal horse, as it grew larger, would be expected to consume less energy per kg. than a foetal rabbit. The fact, as shown by Brody, that a foetus of large birth weight tends to cost more, in Calories per kg., than a foetus of small birth weight, strongly suggests the presence of maternal "overhead charges."

It would be unwise, however, to read too much into this graph, because, owing to its double logarithmic basis, points off the line are much more at variance with theory than they appear (compare Table II). Moreover, the particular method chosen of formalising the experimental data is quite empirical. This does not invalidate our previous deductions regarding maternal metabolism, but a different "law" might have emerged if the data had been used in another way.

III. WATER EXCHANGE

The fluid of the body is now conventionally divided into three "compartments"—the blood plasma, the interstitial or tissue fluid, and the intracellular fluid (Fig. 18. 4). In man, their volumes are about 3 l., 14 l. and 29 l., respectively, and their compositions are indicated in Fig. 18. 5. Intracellular fluid contains a different selection of ions from either of the other two, which are similar in composition and often referred to collectively as extracellular fluid. The fundamental barrier is, therefore, the cell membrane, and osmotic equilibrium between intra- and extracellular fluid is maintained by the transfer of water and perhaps bicarbonate ions, to which the membrane is permeable. The blood stream may be regarded as an extension of the interstitial fluid compartment, which it serves (a) by keeping it thoroughly stirred and (b) by bringing it into communication with the external environment.

The stirring is made possible by the fact that, although the capillary walls are freely permeable to all dissolved non-protein constituents of the plasma, their lack (or relative lack) of permeability to plasma proteins enables the latter to hold

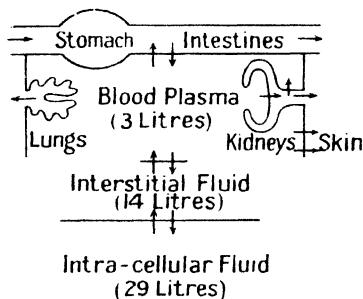


Fig. 18. 4—Diagrammatic illustration of the three fluid compartments. The figures in parentheses have been inserted to show the volume of water in each compartment of a subject weighing 70 Kg., whose total water content is assumed to be 46 litres (two-thirds of the body weight). (From Gregersen, 1941 (after Gamble).)

(Brody, 1938)

DATA ON THE RELATION BETWEEN THE HEAT INCREMENT OF GESTATION AND BIRTH WEIGHT OF OFFSPRING

Species and Breed	No of Animals in Average	Weight of Newborn Individual or Litter Kgs	Estimated Surface Area of Newborn Sq metres	Mother's Weight gain during Pregnancy Kgs	Heat Increment of Gestation		Gestation Period Days	No in Litter	Source
					Total Cal	Cal kg Birth Weight			
Percheron Horse	1	74.0	505	13 0	591	7,986	340		ginal
Holstein Cows	16	40.8	197	94 0	353,400	8,662	283		"
Hereford Cows	3	31.8	040	45 0	304,500	9,575	283		"
Jersey Cows	18	25.4	918	44 0	228,300	8,988	283		"
Duroc-Jersey Swine	6	6.5		9 0	42,120	6,480	114		"
Duroc-Jersey Swine	1	8.1		10 0	65,520	8,089	114		"
Duroc-Jersey Swine	1	4.3			25,000	5,930	114		"
Duroc-Jersey Swine	1	2.7		6.0	9,200	3,407			"
Dorset Sheep	1	5.0	0.300	6.0	23,800	4,755	50		"
Dorset Sheep	1	3.7	0.251	5.5	21,600	5,838	50		"
Human	1	3.8	0.251	8.3	22,540	5,932	280	1	Root and Root Sandiford and Wheeler
Human	1	3.6	0.242	7.7	21,805	6,057	280	1	Original
Goat	1	7.7	0.455	.5	53,300	6,922	150	2	
Goat	1	3.2*	0.203	.8	37,300	11,660	150	1	"
Goat	1	5.2*	0.347	1.2	57,800	11,120	150	2	"
Rat	1	0.0225		—	38.7	1,724	21	5	"
Rat	1	0.0135		—	38.8	2,874	21	3	"
Rat	1	0.058	—	0.016	88.3	1,522	21	12	"
Rat	1	0.050	—	0.014	57.0	1,140	21	9	"
Rat	1	0.059	—	0.024	94.8	1,607	21	13	"
Rat	1	0.052	—	0.026	84.8	1,631	21	12	"
Domestic Fowl †	—	—	0.116	—	19.0	501	21	—	Average various sources

* Estimated on basis of average values.

† Omitted from correlation.

fluid against the intravascular pressure which keeps the blood in motion. Since the mechanical pressure at the proximal end of a capillary vessel is greater than the colloid osmotic pressure of the plasma, it is possible for fluid to be forced out of the capillary at the beginning of its course, while an equivalent amount is returned at the end when the mechanical pressure has fallen below the osmotic pressure of the plasma proteins. It follows that if for any reason the protein concentration of the plasma should fall, not only is water likely to leak out in the tissues, but the normal exchanges between plasma and interstitial fluid may be retarded.

Contact with the external environment is made by the blood through the lungs and skin, which need not greatly concern us, and through the alimentary tract and kidneys, which for our present purpose are more important. Here, water and soluble constituents are gained and lost in considerable quantities, the normal result being that the composition, and in particular the pH and osmotic pressure, of the extracellular fluid are kept constant. Salt retention by the kidneys will lead to the retention of that volume of water which will maintain the osmotic pressure of the intracellular fluid will be drawn upon for water, and the cells will suffer dehydration. Although at first sight it may seem that the final volume of extracellular fluid so determined does not matter, this is by no means true. For instance, in the salt-retention just described the plasma proteins would be diluted, with a consequent redistribution of water between plasma and interstitial fluid, and a less efficient interchange between them. Furthermore, the actual excess of fluid would become embarrassing in a variety of ways.

The evidence on water distribution during pregnancy is quantitatively incomplete, because continuous water-balance experiments are difficult to make, and data which can be obtained from numbers of separate observations do not lend themselves to very accurate analysis. Most of the observations have been made on women, and are presented and discussed in a review by Chesley (1944). Other references to the original literature are given by Harding (1925), Adolph

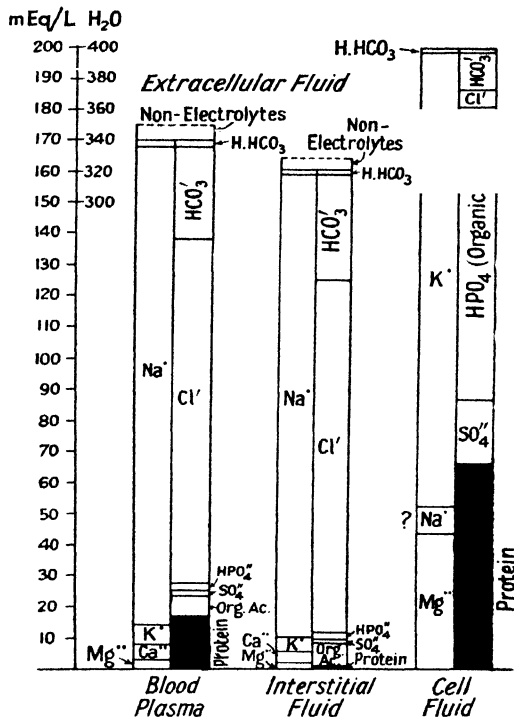


Fig. 18. 5—Diagram showing the chief chemical constituents of the three fluid compartments. The height of the left half of each column indicates the total concentration of cations, that of the right half the concentration of anions. Both are expressed in milliequivalents per litre of water. Note that the chloride and sodium values in cell fluid are questioned. It is probable that at least in muscle the intracellular phase contains some sodium but no chloride. (From Gregersen, 1941 (after Gamble).)

(1933), Dieckmann and Wegner (1934 a, d), Albers (1939), Peters (1944) and Taylor, Warner and Welsh (1943).

An increase in body weight, particularly if it is large, rapid and easily lost again, is often assumed to be due to water. This is, indeed, the most likely explanation, but likelihood is far from certainty. In view of the inconclusive nature of nearly every line of evidence—taken by itself—that the mother retains water during pregnancy, her increase in weight, over and above that of the products of conception and of the hypertrophied accessory sex organs, is often given more significance than is at present justified. It is quite clear from Chesley's review, of which the gain in weight of women during pregnancy is the basis, that the additional weight can be accounted for many times over and is not necessarily due to water. Chesley has made averages of a large number of figures, obtained from the literature, and from his own observations, and his assessment of the situation is worth summarising here in order to place arguments from body weight in perspective. It is somewhat as follows :—

Average total gain in weight (11,960 cases)	24 lb. (10·8 kg.)
Average of averages of different observers	23·1 lb. (10·4 kg.)
Range of averages	13·3–37·4 lb. (6–16·8 kg.)
(14 out of 19 averages are between 20 and 25 lb. or 9 and 11·3 kg.)	

For about half the cases enough data are available to find the standard deviations, which are all about 45 per cent. of the means. If this is true for all cases, two-thirds of normal women will gain between 13 and 35 lb. (5·8 and 15·8 kg.) during pregnancy—on the average, 2·5 lb. (1·1 kg.) in the first third, 10·8 lb. (4·9 kg.) in the second third and 11·2 lb. (5 kg.) in the last third of pregnancy. A large proportion of individuals suffer a loss of weight of up to 2·5 lb. (1 kg.) during the last three days before parturition.

The average weight of the baby, placenta and amniotic fluid is 11·2 lb. (5 kg.), so there is a residual gain in weight of 12·8 lb. (5·75 kg.), which is 10 per cent. of the average pre-pregnancy weight of 128 lb. (57·5 kg.). Of this the increased size of the uterus will account for about 2·5 lb. (1·1 kg.) and the breasts for 2 to 4 lb. (0·8 to 1·9 kg.). This still leaves 7 lb. (3·2 kg.) unaccounted for.

It must be remembered that this figure has been arrived at by the manipulation of various averages and that individuals vary within wide limits. For instance, about 10 per cent. of women probably finish up with a net loss of weight rather than a net gain, and in others the gain in weight to be accounted for will be very much larger than 7 lb. (3·2 kg.). There can be little doubt, however, that this average residual gain represents something real. Table III shows that in guinea-pigs, rats and mice, it averages 11·6 per cent. of the body weight. Mice in which the foetuses have been destroyed but the placentas allowed to survive maintain the weight already added until the placentas are delivered, and monkeys which have been treated similarly actually resume their increase of weight (Fig. 18, 1) until parturition. The increase in weight could be due to fat, protein or water. Chesley neglects fat for lack of data, but shows there is sufficient nitrogen retention to account for as much as 20 lb. (9 kg.) gain in weight if the protein were built up into the form of protoplasm. If it were stored in some more concentrated form it might only account for 4 lb. (1·8 kg.) outside the reproductive tract,

TABLE III
(From Chesley, 1944)

NET GAIN IN WEIGHT ACCRUING TO MATERNAL TISSUES DURING PREGNANCY IN ANIMALS
OTHER THAN WOMAN

Author.	Animal.	No. of Animals.	Percentage gain Maternal Tissues during Pregnancy.
Minot	Guinea-Pigs	66	15
Herring	Rats	9	no gain
Hartwell	"	6 (40 pregs.)	13
Hain	"	16	12-15
Abramson	"	44	6
Brühl and Vogel	Mice	4	16
Poo, Lew and Addis	Rats	20	12
Totals and Mean		199	11.6

but although there is considerable evidence for certain organs acquiring an increased weight, there is no evidence anywhere of a concentration of protein.

The case for water retention must, therefore, rest on more direct evidence, since additional weight can be accounted for by protein. This evidence may be summarised as follows :—

(1) There is a marked post-partum diuresis accompanied by a rapid loss of weight. Loss of weight in mice and monkeys also occurs when they deliver placentas which have been retained following elimination of the foetuses. The only adverse comment on this type of evidence is that if protein stored in the form of protoplasm were rapidly metabolised it would also be accompanied by diuresis.

(2) After the diuresis the tissues are atonic and flaccid, and a shrinkage in the circumferential measurements of arms, legs and neck has been recorded.

(3) There is a positive sodium balance during most of pregnancy (Table IV). It will be seen from the last column of Table IV, however, that if all the sodium were ionised, the retention of sufficient water to keep the osmotic pressure of the tissue fluids normal would more than account for the total average gain in weight. The suggested rationalisation that part of the sodium may be retained in some osmotically inactive form, perhaps in combination with protein, somewhat begs the question of using the sodium balance to support the evidence for water retention, but there is a certain parallelism between the fluctuations of the Na and Cl balances (Thompson and Pommerenke, 1939) and between Na and water retention (Freyberg, Reekie and Folsome, 1938), and it seems wise to assume that at any rate some of the sodium is osmotically active.

TABLE IV
 NA RETENTION DURING NORMAL PREGNANCY
 (From Chesley, 1944)

The last column is calculated from the assumption that all Na is held extracellularly, is osmotically active and that its concentration in extracellular water is 336 mg./100 ml.

Author.	Cases.	Period in Pregnancy.	Mean daily retention Na, gm.	Total retention gm. per wk.	Calculated gain in extracellular water; litres per wk.
Coons <i>et al.</i>	5	Near end	1.26	8.81	2.620
Hummel <i>et al.</i>	1	Last 21 weeks	0.56	3.92	1.167
Freyberg <i>et al.</i>	1	Last 70 days	0.231	1.61	0.473
Thompson and Pommerenke	1	In 4th and 8th months	0.44	3.08	0.917
Taylor <i>et al.</i>	4	Last 13-23 days	0.267	1.87	0.557
			0.440	3.08	0.917
Taylor <i>et al.</i>	3	1st 10 days, post-partum	0.489	Total loss 1st 10 days post-par-tum 4.89	Loss 1st 10 days post-par-tum 1.455

Owing to the impermeability to sodium of the cell membrane, retained NaCl with its complement of water, would be confined to the extracellular compartments.

(4) All observers are agreed that the total blood volume is increased by approximately 20 to 30 per cent. and that the plasma volume is increased much more than the red cell volume, so that a superficial blood examination may suggest anaemia. The increase in blood volume would account for about 3 lb. (1.4 kg.) in weight (Fig. 18. 6).

Using the dilution of injected thiocyanate to estimate the total volume of extracellular water, Chesley (1943) found an average gain during pregnancy of 13.85 lb. (6.2 kg.), of which 9 to 10 lb. (4 to 4.5 kg.) would be maternal. Of this, 2.9 lb. (1.3 kg.) would be in the blood and 1.6 lb. (0.7 kg.) in the uterus and breasts. Chesley (1944) seems a little doubtful about the significance of the interstitial fluid residue of 4 to 5 lb. (1.8 to 2.3 kg.), but this estimate seems based on as good, if not better evidence than some of the previous estimates from average measurements.

(5) If the plasma proteins were supplemented proportionately to the increased load of fluid, and provided there was no change in the relation between capillary blood pressure and tissue fluid pressure, then the ratio of tissue fluid to plasma should remain as in the normal individual. The plasma proteins are, however, found in diminished concentration in the plasma, albumin more so than globulin, although there is an increase in the *absolute* quantity of plasma protein in the body. (Fig. 18. 7, Albers, 1939, and Chesley's review). (Dieckmann and Wegner, 1934b, Albers, 1939, Mull and Bill, 1945, and others have shown that the haemoglobin is

also increased in total amount in spite of the fall in its final concentration.) The fall in colloid osmotic pressure of the plasma which ensues is from 15-20 per cent. and means that the blood will retain less than its fair share of the additional fluid.

Goudsmit and Louis (1942) state that in normal individuals they find the total extracellular fluid to be increased by 3.8 per cent. of the body weight when the plasma proteins are decreased by 1 g. per 100 cc. Unfortunately, they give no

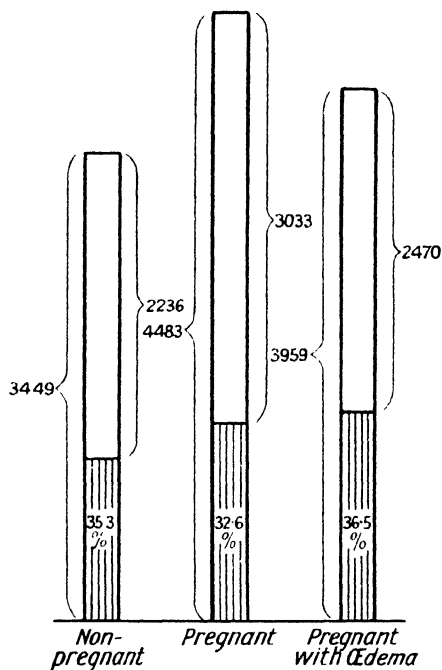


Fig. 18. 6—The change in blood volume during pregnancy. The figures against the covering brackets indicate blood and plasma volumes respectively in cubic cm. The lower shaded portions indicate the percentages of blood cells. (From Albers, 1939.)

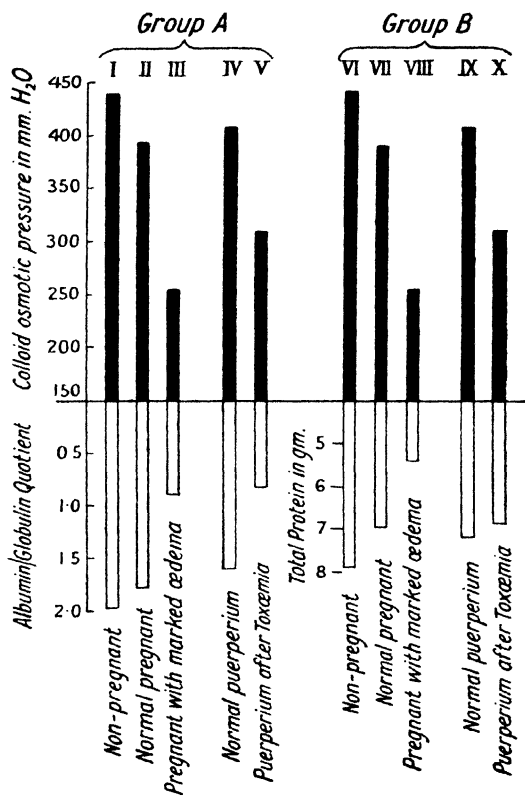


Fig. 18. 7—Colloid-osmotic pressure, total protein and albumin-globulin quotient in non-pregnant and pregnant women and in patients suffering from toxæmia. (From Albers, 1939.)

details, but evidently a dilute plasma allows water to accumulate in the interstitial fluid compartment. Melnick and Cowgill (1937) have shown that pregnancy in the dog reduces the rate at which the liver can regenerate plasma proteins, when these are continually removed, and Stewart and Rourke (1942) have demonstrated the normal response to a large increase of 80 per cent. in the volume of extracellular fluid. To do this, they infused a patient with 6.5 l. of 0.9 per cent. NaCl solution per day for 4 days. The total quantity of serum protein rose from 233 to 283 g. during this period, the concentration falling from about 8 g. to about 6 g. per 100 cc. The distribution of the water load was such as to increase the

plasma volume by 60 per cent. and that of the interstitial fluid compartment by 90 per cent. This seems a fairly brisk regenerative response on the part of the serum protein, and while the experiment illustrates well the way in which fluid distributes itself in the circumstances, it also suggests that the liver would normally have the inherent capacity to respond fully over a longer period to the moderate water load of pregnancy. Whether its failure to do so is due to the deficiency revealed by Melnick and Cowgill, or whether it is part of the syndrome of pregnancy, remains to be seen.

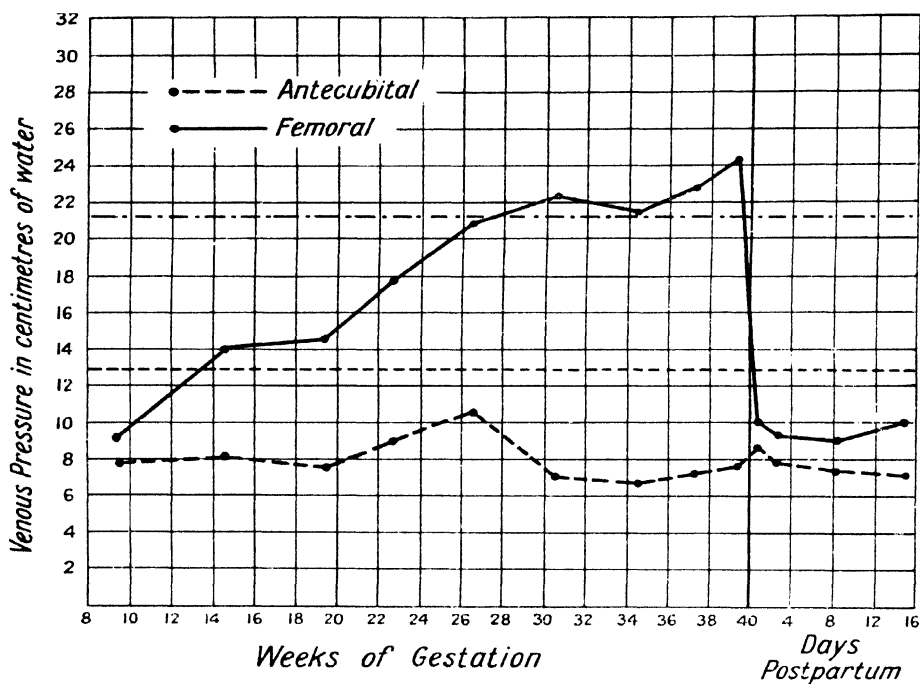


Fig. 18. 8—Showing the average course of antecubital and femoral venous pressure throughout normal pregnancy and the immediate puerperium, based on 173 antecubital and 227 femoral determinations in 160 pregnancies. The lower straight dotted line represents the upper limit of normal variation for antecubital venous pressure and the upper broken line the same for femoral venous pressure (i.e. control mean + 3 S.D.). (From McLennan, 1943a.)

(6) A second factor which tends to throw the load on to the interstitial fluid compartment is an increased venous pressure. Although little or no increase is revealed by direct measurement in the antecubital vein (Burwell, 1938a, McLennan, 1943a, Albers, 1939), there is a rise in pressure in the femoral vein during pregnancy from an average of 91 mm. of water to one of 244 mm. in the supine position (Burwell, 1938a, McLennan, 1943a, Fig. 18. 8). The latter is attributed to external pressure on the abdominal veins by the enlarged uterus, or to internal resistance set up by the copious flow of blood from the uterus. If the placental circulation represents an arterio-venous anastomosis, this blood would be delivered into the veins at a pressure higher than normal. Quite apart from any diminution of

colloid osmotic pressure in the blood, the observed hydrostatic pressures in the latter part of pregnancy are sufficient to increase the amount of tissue fluid in the legs, which represent 30 per cent. of the body weight.

Saphenous pressure, measured standing, averages 938 mm. water, which must lead to the temporary retention of a considerable volume of fluid. The water accumulating as a result of standing is, however, almost certainly taken back into the blood stream and excreted during recumbency: it is well known that the volume of urine excreted a night is, in pregnancy, as great as or greater than that excreted during the day.

(7) A third factor, reinforcing the effects of lowered colloid osmotic pressure and increased filtration pressure in the capillaries may be an increased permeability of the walls of these vessels, but this is difficult to prove. By producing venous congestion in the forearm it is possible to measure the volume of the arm before and after (McLennan, 1943b), or the increase in concentration of the venous blood with respect to blood cells and protein during, congestion (Albers, 1939). It is not easy to assess the resistance to filtration offered by the already increased content of the interstitial fluid compartment, or the facilitation of filtration by the decreased osmotic pressure of the plasma. McLennan is clearly doubtful of the significance of the increase of transudate over and above the normal which he found. Albers, however, found that 83 per cent. of pregnant women, irrespective of the period of pregnancy, lost fluid from the blood stream more readily than normal persons under the same conditions, and that 62 per cent. also lost plasma protein. For the reasons given above, it is a serious matter from the circulatory and metabolic point of view if the capillary walls become permeable to protein.

In spite of the factors tending to divert the waterload from plasma to interstitial fluid, the averages of actual determinations seem to show that of a total of 4.5 l. of additional extracellular water belonging exclusively to the mother, as much as 1.3 l. is in the plasma. This proportion is widely different from the normal distribution of 3 out of 17 l. (Fig. 18. 4). Even if the whole of the additional extracellular fluid of pregnancy (6.2 l.) is assumed to belong to the mother, the proportion accounted for by the plasma is high instead of low. It follows that either the number of cases studied has been insufficient to yield representative figures, or that the assumptions on which the determinations of blood volume or extracellular fluid are made are invalid during pregnancy, or (improbably) that an unknown factor of some other kind is operative.

(8) The generally accepted view of events in the puerperium is that of Taylor, Warner and Welsh (1943): "During labour and immediately afterwards, the blood becomes more concentrated. This is in part due to blood loss, partly to the effects of muscular effort and dehydration, and perhaps in part to a redistribution of water as a result of the disappearance of the placental circulation. This period of blood concentration is of short duration, however, for within forty-eight hours the plasma volume again rises and immediately thereafter diuresis commences. Large quantities of sodium appear in the urine during the next few days, and there is a considerable loss of available extracellular water." There can be little doubt of the main facts, which are well supported, though sodium retention has been described during the puerperium. Albers, however, has advanced an additional and most plausible explanation of the blood concentration following

labour, which is illustrated in Fig. 18. 9 from his own measurements. During labour the venous pressure rises considerably with each pain, increasing with their severity, until it is maintained at a high level between the rapidly following pains. During actual delivery it may rise to extreme heights. Albers believes that a

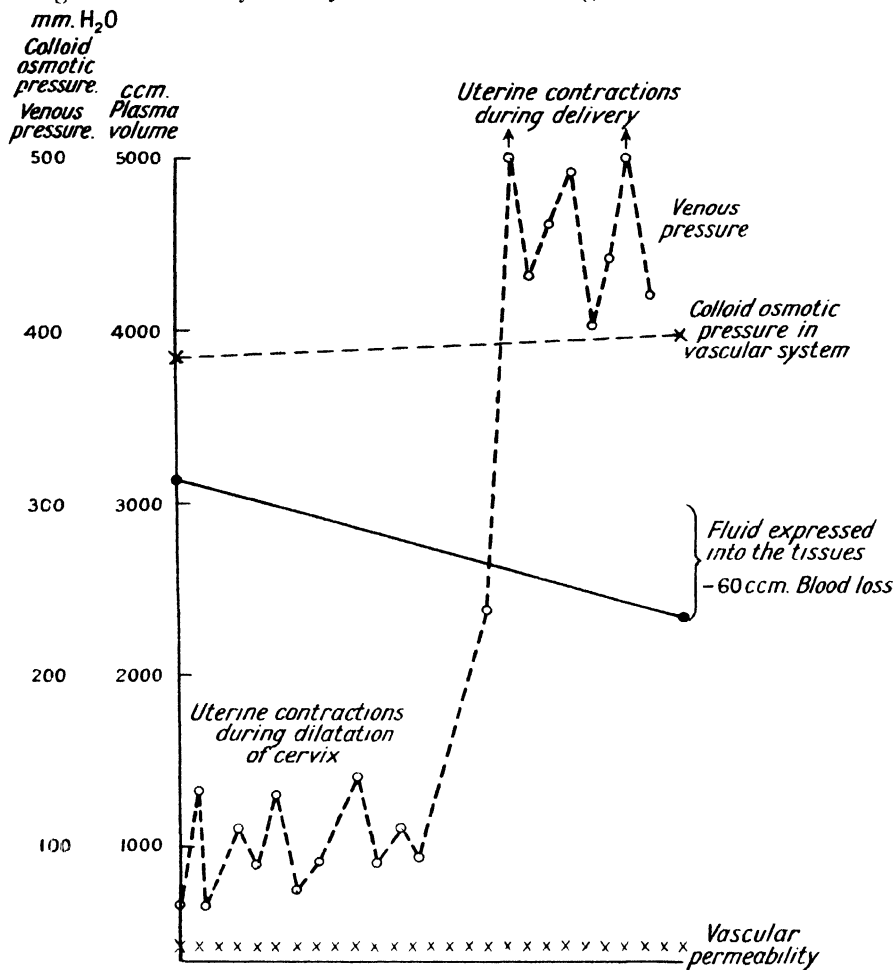


Fig. 18. 9.—Factors which influence the transfer of water from blood to tissues during parturition. (From Albers, 1939.)

considerable quantity of fluid is then forced out of the blood stream and into the interstitial fluid.

(9) The high concentration of oestrogen (Zuckerman, Palmer and Bourne, 1939) and progesterone in the body fluids during pregnancy may be concerned in the retention of sodium and water. Taylor, Warner and Welsh point out the association in various animals of sodium and water retention with either naturally produced or injected oestrogen. They have shown that the change in sodium balance

before and after labour follows the change in the urinary concentration of oestrogen and pregnanediol, and they achieved some success in arresting the negative sodium balance of the puerperium by injecting oestradiol benzoate, oestradiol or progesterone. Alteration in the endocrine balance is a likely source of the observed changes and of their variability from individual to individual. If sodium retention is the initial process, it seems more reasonable to attribute it to such a cause than to a primary change in the kidney.

In spite of this chain of evidence, it must be admitted that analyses by various investigators of tissues such as the liver, muscle and skin of pregnant animals has revealed little or no increase in their water content (e.g. Hewitt and Van Liere, 1941). The various data on the whole subject of water retention are, quantitatively, not very satisfying.

IV. MATERIAL EXCHANGES

We have remarked that from the material point of view the mother may be better off at the end of pregnancy than at the beginning, in spite of having provided the building materials and fuel for the foetus. We have also seen in the last section that there is a definite tendency for the mother to gain in weight, though it seems that this may be accounted for many times over and may in any event be due to an excess of water. Obviously, the only satisfactory criterion is the gain and loss of significant and measurable chemical substances.

Carbohydrate and fat are elusive, owing to the inter-convertibility of the carbon, hydrogen and oxygen in the molecules of the three foodstuffs, but the utilisation of protein and certain minerals can be followed. A positive N-balance (more in the food than in the excreta) indicates the building of new protoplasm, the only known form in which nitrogen can be stored. A negative N-balance indicates the breakdown of body tissue, such as occurs in starvation. Amino-acids from the autolysis of less essential tissues (e.g. voluntary muscle) pass into the blood stream, and those amino-acids which are not built up into the protoplasm of the more essential tissues (e.g. cardiac muscle) are oxidised, with the inevitable loss of N in the urine. The theoretical possibility—at one time a widely held belief—that the protein of the foetus is manufactured at the expense of the maternal tissues demands an investigation of the N exchange during pregnancy.

We have seen that the total energy metabolism during pregnancy is increased, and if the diet were low the burden of this increase might fall on the maternal protein, quite apart from the specific foetal requirements for N. We shall assume fortuitous factors of this kind to be eliminated in the experimental investigations to be considered.

The mere existence of a positive balance of an element during pregnancy does not constitute proof that foetal demands are being satisfied without detriment to the mother. Only if the positive balance is at least equal to the foetal requirements may we assume that the mother is not suffering. The difficulty is to know the foetal requirement. The gain or loss of an element in pregnancy can be assessed in three ways :

(1) If the balance of an element E be followed throughout pregnancy and the foetus or foetuses killed at the end and analysed, it is possible to say at once whether the maternal tissues have gained or lost. This experiment has rarely been made, and would be a practical proposition for few species.

(2) One or other of the determinations of (1) may be replaced by an estimate. (a) Instead of an actual determination of the E-content of the foetus which, as in man, it may not be possible to sacrifice, an E-content may be assumed, based on the average content of a number of dead foetuses of the species. (b) Instead of a continuous E-balance being made on the mother throughout pregnancy,

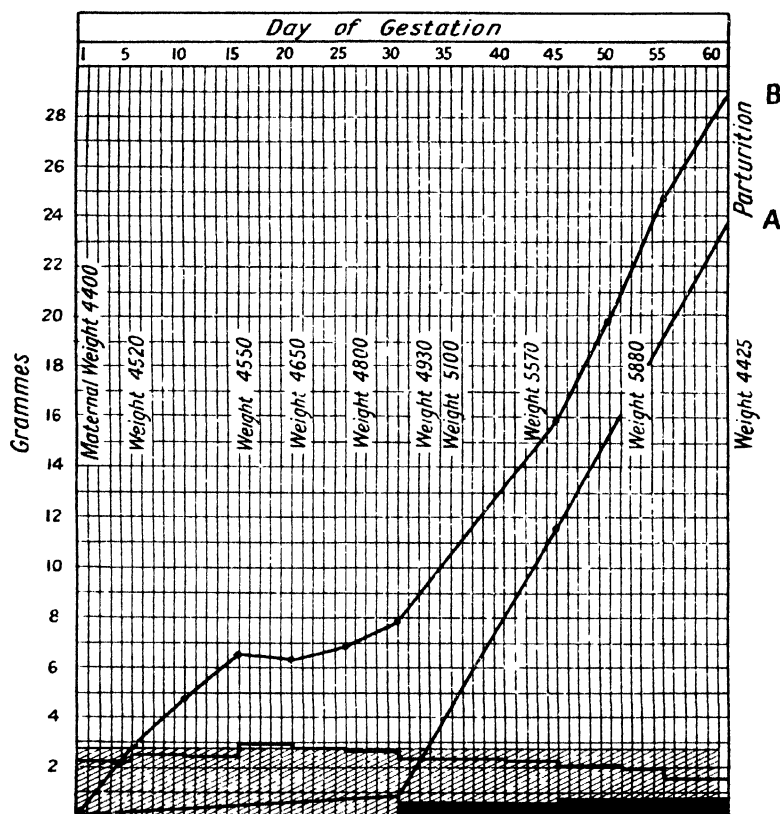


Fig. 78. 10- - N-balance of a bitch during the whole of gestation. The height of the grey area represents the daily absorption of N, and the superimposed horizontal lines the daily excretion. The cumulative result of the difference is shown by curve B. The height of the black area indicates the daily diversion of N to the foetus, the cumulative loss in this direction being shown by line A. (From Bar and Daunay, 1905.)

balances may be carried out for a specified number of days at frequent intervals during pregnancy and the balance in the intervals estimated by interpolation.

(3) Finally, if average foetal weights at all periods of pregnancy are available, a continuous balance can be carried out over a part of pregnancy, and the result compared with the estimated gain by the foetus during that period.

In order fairly to represent the difficulty in obtaining the necessary facts about the exchange of N and other elements, no reference has been made to the accretion of purely maternal tissue which occurs in the uterus and mammary

glands as a result of pregnancy. This cannot strictly be regarded as maternal gain, and the amount of E which it represents must be estimated and subtracted from the credit side of the account.

The nitrogen balance.

With the work of Bar and Daunay (1905) and Murlin (1908, 1910) on dogs, it was established that nitrogen retention took place during pregnancy. These were not the first N-balance experiments which had been made, but previous ones were unreliable (see Lochhead and Cramer, 1922).

Bar and Daunay gave their bitches a constant daily ration of a mixed diet of known N-content, and periodically analysed all the excreta, with the result that they were able to plot the daily retention (or loss) of N on a cumulative curve (Fig. 18. 10). At the end of gestation, the pups (with placentae) were killed and their N-content determined. Two further bitches were sacrificed at the 30th and 45th days of pregnancy respectively, and it was ascertained that for every gramme of N present in a full-term foetus, 0.35 g. was already fixed in a 30-day and 0.45 g. in a 45-day embryo. All the mothers were originally of the same weight and served by the same dog, and the 4-point "curve" of foetal N-requirements constructed from these data for each experiment was probably not far from the truth. The shape of the curve (see Fig. 18. 10) strengthens this impression—the "correct" point of inflexion at which the foetal-N curve begins to rise rapidly may be a little earlier than depicted, but the positions of the two succeeding points make it unlikely to be later.

Similar experiments, in some ways more and in some ways less complete than Bar's, were made independently by Murlin (1910). In the two of his dogs in which pregnancy was completed, one showed a gain, the other a loss of N as a result of pregnancy, but there is some doubt as to the calorific adequacy of the diet during the early weeks. He gives an interesting table, however, summarising the results of his own and others' work in which one feature constantly reappears, irrespective of the adequacy of the experiments in other respects. This is a period of negative N-balance in the early part of pregnancy (Table V). Fig. 18. 10 illustrates the second experiment of Bar and Daunay—upon the animal in Table V in which the balance just fails to become negative—and shows that even in this dog there is a pronounced check in the rate of nitrogen storage. No more than speculations have been offered in explanation of the phenomenon, though the one which attributes it to the action of proteolytic enzymes from the trophoblast is supported by the existence of an increased concentration of anti-trypsin in the blood stream.

During the period of deficient storage, the dogs rejected much of their food and showed signs of sickness. The obvious parallel between this and morning sickness in women has been drawn, but no special disturbance of N-equilibrium has been emphasised at this time in women. The dip in the BMR curve for women (Fig. 18. 2) which does not coincide with the period of morning sickness is just as likely to be an associated phenomenon. In the BMR determinations of Brody and his school on various animals, there is also often a dip in the curve early in pregnancy. Perhaps the most noteworthy feature of the N-storage shown in Fig. 18. 10 is that it begins with the onset of pregnancy, and becomes quite large at a time when the foetal demands are yet negligible. This may often,

TABLE V
NITROGEN BALANCE IN PREGNANT BITCHES
(Murlin, 1910)

Date.	Author.	No. of exp.	Food Cal. and gm. N per kgm.	N balance by weeks of the pregnancy.								
				1	2	3	4	5	6	7	8	9
1891	Hagemann	I	82*	1.0*	-	-	-	-	+	+	+	+
	"	II	110	0.8	-	-	-	+	+	aborted		
1903	Jägerroos	I	75	0.8	+	+	-	-	+	+	+	+
	"	II	106	1.84	+	-	-	+	+	+	aborted	
	"	III	75	1.31	+	+	+	+		+	+	+
	"	IV	70	0.2		+	-				aborted	
1907	Bar	I	112	0.64	+	+	+	+	+	+	+	+
	"	II	80	0.66	+	+	+	+	+	+	+	+
	"	III	116	0.67	+	+	-	+	+	+	+	+
1910	Murlin	I	70	0.75	-			-	+	+	+	+
	"	II	70	0.64				-		+	+	+
	"	III	70	0.75	+	+	not continued			+	+	+
	"	IV	70	0.8	+	+	-	-	-	+	not continued	

* These figures are based on the weight of the dog always at the beginning of the experiment.

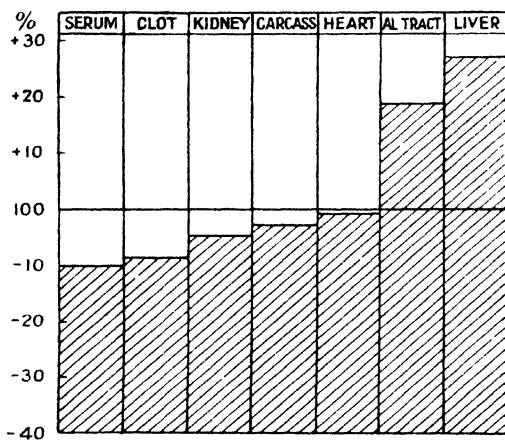


Fig. 18. 11—Distribution of protein in pregnant rats relative to its distribution in control non-pregnant rats. The organ or tissue protein per 100 g. total protein in the controls is plotted as 100, and the protein per 100 g. maternal protein of the corresponding organ or tissue of the pregnant animals is shown as a percentage increase or decrease. (From Poo, Lew and

however, be counterbalanced by the loss incurred during the succeeding period, and the general tendency of investigators is to emphasise the paucity of N-retention early in pregnancy.

A series of careful experiments on pregnant sows by Evans (1929) brings out this point, though one out of the four sows selected to study the question showed excellent early N-storage. Crowther and Woodman (1922) who studied the dairy cow found that in this animal the rate of storage also became pronounced only during the month preceding parturition, and in a review of the literature, Harding (1925) is struck by the small positive, or actually negative balances in early gestation. In spite of this, there can be no doubt that mothers of all species investigated, if adequately fed, finish

pregnancy with a net gain of nitrogen to themselves. Thus Evans' sows stored an average of 1439 g. of N, of which only 233 g. was required for the foetuses. Although some N would be included in the adnexa and the uterine and mammary growth of the mother, it is obvious that considerable other maternal

storage had taken place. The storage is commonly regarded as preparation for lactation.

An original approach to the question of N-storage has been made by Poo, Lew and Addis (1939) and Poo, Lew, Lee and Addis (1940), who claim to estimate protein directly in tissues. They find that pregnant rats killed on the 18th day of gestation have accumulated twice as much protein as control rats, but as their total weight increases even more quickly, the final concentration of protein in all tissues is diminished. The diminution is greater compared with the controls, in serum than in the liver, kidney, heart or alimentary tract. If, however, the protein in each organ is expressed as a percentage of the total protein (excluding uterus and products of conception) it is found that the distribution of protein in the pregnant is different from that in the non-pregnant animal. In Fig. 18. 11 the share of the total protein allotted in pregnancy to each organ is expressed as a figure so many per cent. greater or less than the normal share as shown by the control non-pregnant animals. The liver and the alimentary tract seem to receive very preferential treatment, and it is to be assumed (on account of the absolute concentrations found) that much of the retained water goes to the same destinations. On a protein-poor diet, the storage is less throughout.

Rats on a protein-free diet from the 13th day of pregnancy onwards produce litters, but at the expense of the mother (Seegers, 1937, 1938). The maternal protein is evidently very suitable for the manufacture of foetal protein because there is extraordinarily little wastage of nitrogen. It is generally assumed that in tissue breakdown, the amino-acids have to run the gauntlet of the liver, where some, even though suitable for building up into other tissues, are broken down and oxidised with the formation of urea. The fact that this expected loss of N does not take place during starvation in pregnancy may have something to do

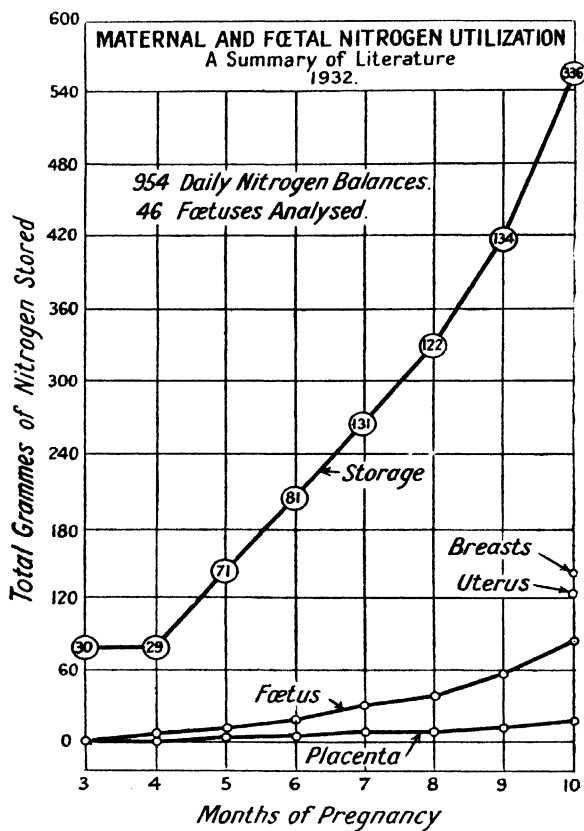


Fig. 18. 12.- Showing the maternal and foetal storage of nitrogen, and the amounts used by the breasts, placenta and uterus, from the 3rd to the 10th month of pregnancy. The figures enclosed in the circles represent the number of days of metabolic observations. (From Macy and Hunscher, 1934.)

with the fact that the substance of the maternal liver is itself an important source of nitrogen for the foetus, and the breakdown of liver protein may be incompatible with the oxidation of amino-acids by that organ. Harding (1925) comments on "the economy with which the pregnant organism utilises valuable protein."

Numerous investigators have confirmed the early work of Hoffstrom (1910)

MATERNAL MINERAL STORAGE

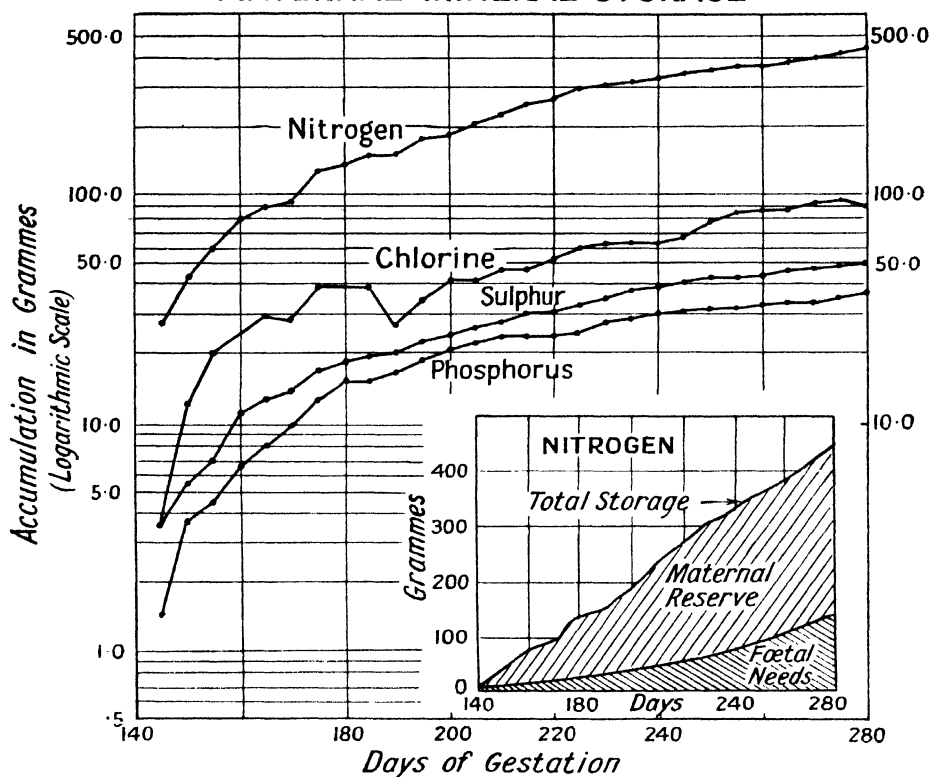


Fig. 18. 13—Shows the continuous storage of nitrogen, chlorine, sulphur and phosphorus during the final 145 days of gestation. The days are plotted arithmetically and the maternal accumulation in grammes logarithmically, thus illustrating the absolute values and the relative rates of change with the progression of foetal development. The inset shows the large determined maternal storage of nitrogen that has been laid down by the mother in excess of the foetal needs. (From Hummel, Sternberger, Hunscher and Macy, 1936.)

and Wilson (1916) on the N-balance in pregnant women. The recent comprehensive investigations of Macy and her colleagues have left nothing to chance, and their careful compilation of data from the literature, and their equally careful continuous balances made on pregnant women over periods of months, have placed the elementary facts beyond doubt. Fig. 18. 12 epitomises the position with regard to N-storage, based on the findings of various workers, up to 1932. Fig. 18. 13 shows the accumulation of N, Cl, S and P in one pregnant woman (Hummel, Sternberger, Hunscher and Macy, 1936) during the last 5 lunar months of

pregnancy. (The vertical logarithmic scale has the effect of showing each week's increment as a proportion of the previous week's total.) In the inset, the N-accumulation is plotted on a linear scale, and contrasted with the presumed foetal requirements. There is no doubt that, on an adequate diet, considerable storage of N takes place.

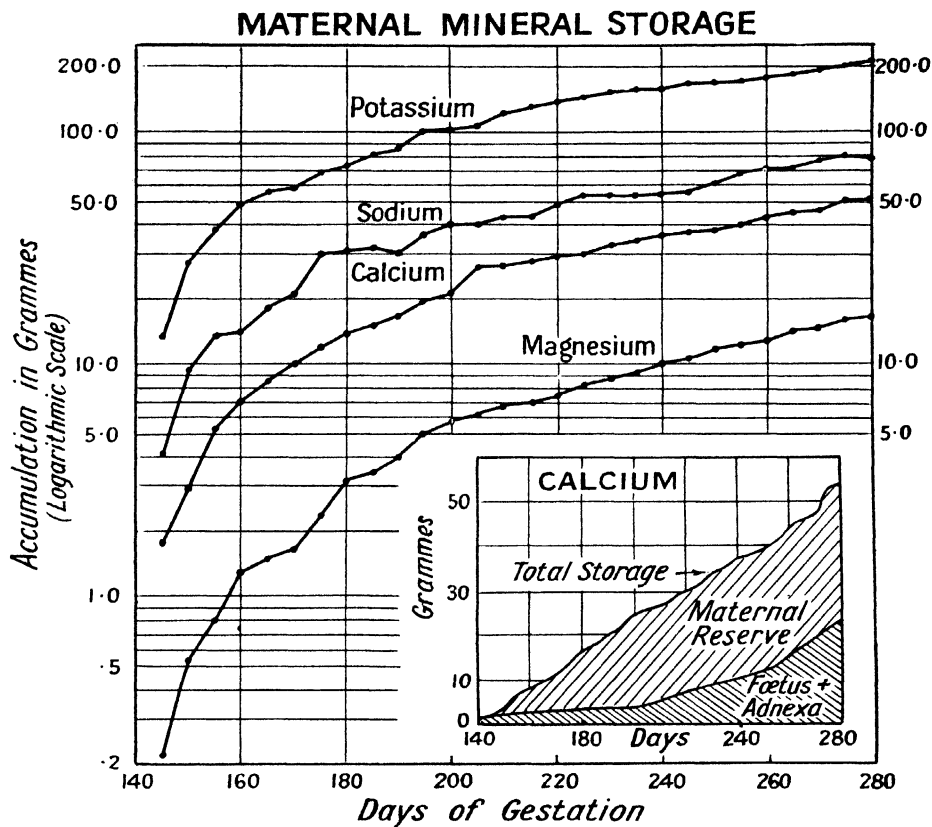


Fig. 18. 14—Shows the continuous maternal storage of potassium, sodium, calcium and magnesium during the final 145 days of gestation. The days are plotted arithmetically and the maternal accumulation in grammes logarithmically, thus illustrating not only the absolute values but the relative rates of change with the progression of foetal development. The inset shows the large determined maternal reserve of calcium that is stored beyond the estimated needs of the foetus and its adnexa. (From Hummel, Sternberger, Hunscher and Macy, 1936.)

The mineral balance.

Figs. 18. 13 and 14 show the cumulative storage of Cl, P, K, Na, Ca and Mg in a well-nourished pregnant woman (Hummel, Sternberger, Hunscher and Macy, 1936). The inset curve shows how greatly storage exceeds the foetal requirements in the case of Ca, and reference to the average composition of 96 foetuses at various ages (Givens and Macy, 1933) indicate that the Mg and total ash of the foetus also

lag far behind the maternal storage. These results, although obtained from a single individual are important for several reasons. The woman's history was known, previous balance experiments had been made upon her, and this was her fourth experience of pregnancy which was known to cause her no physiological embarrassment. Other reasons are given by the authors for regarding her as a "perfect" subject. No trouble was spared by the investigators, and her diet was virtually unrestricted. The observations are comprehensive enough to reveal a parallel storage of all the elements investigated, a fact which itself lends a certain support to the individual findings.

The same group of workers, however (Hummel, Hunscher, Bates, Bonner and Macy, 1937) point out the influence on storage of the previous diet and state of nutrition of the individual. They say "the metabolic balance method for determining food standards is not precise and cannot be interpreted too freely without further knowledge of the influencing factors, because of such wide physiological variations observed among individuals with highly different nutritive and hereditary backgrounds, and of significant fluctuations of the same individual from time to time when maintained under highly controlled environmental conditions and constant diet."

The "purpose" of the various stores (including those of N) may be to meet the demands of lactation, and of the foetus during the last few weeks of pregnancy, when the current material intake may be insufficient. What is difficult to understand, and what remains unexplained, is that when the pregnant organism is given the appropriate conditions (a full diet) it is in fact capable of laying up these stores. That they bear little or no relation to the immediate demands of the foetus reinforces the idea that the state of pregnancy is a special maternal syndrome, initiated by the products of conception, but not adjusted from moment to moment to their requirements. Barelare and Richter (1938), for instance, have shown that the appetite of pregnant rats for NaCl was so increased that they took twice the normal amount during the first half and three times the normal amount during the second half of pregnancy. During the 10 days post-partum the intake was virtually normal. The animals chose their own food from various containers, and also ate more protein and fat during pregnancy.

Calcium. On account of the danger of Ca deficiency, much attention has been paid to this element and to phosphorus. It is possible that the fall in concentration of Ca and P in the serum as pregnancy advances may be due partly to dilution of the blood. The same may be true of the diminution in serum-Mg described by Wolff and Bourquard (1937). Mull and Bill (1934) confirmed by Bodansky and Duff (1939) point out the interesting fact that serum Ca (but not serum P) is on the average higher during the summer than the winter months. This seasonal difference is superimposed on the effect of pregnancy. Bodansky, Campbell and Ball (1939) show that the incidence of hypocalcaemia (below 9 mg. per cent.) is as high as 23 per cent. at the end of pregnancy, but Dieckmann *et al.* (1944) were unable to demonstrate any increase in the average serum Ca or P by adding these, with or without vitamins A and D, to the diet of their pregnant subjects.

As an example of careful work in revealing unexpected dietary deficiency in pregnancy, followed by experimental investigation, that of Toverud and Toverud (1931) is of great interest. Four-day balances carried out on women in a home for expectant mothers showed that the diet was a "threshold" one for Ca and P.

There was a negative Ca balance in 21 and a negative P balance in 12 out of 27 experiments made in the latter part of pregnancy. Totals of 1.6–2 g. of Ca and 1.8–2 g. P daily were required to correct the balance, but in a few cases in which the dietary deficiency was slight, liberal vitamin D intake alone made the balance positive.

A bitch was then fed, before and during gestation, on a diet low in Ca, P and vitamins A and D. Three-day balances throughout gestation revealed that she lost 10 per cent. of her original Ca content, and 8 days after delivery she developed tetany (serum Ca 5.15 mg. and P 2.92 mg. per 100 cc.). Chemical analysis of metatarsal bones resected respectively before gestation and after lactation showed a great loss of Ca and P. The bones of the puppies were normal to X-ray and microscopic examination, but not to chemical analysis, and those puppies allowed to survive developed rickets on the maternal diet six weeks after weaning. Rickets was later and less marked in the puppies of a normal mother who was given the experimental diet only from the day of delivery, and still further delayed in those given the experimental diet after weaning from a mother on a normal diet (*see also* Sontag, Munsen and Huff, 1936). Repetition of the experiment with the same diet, but with the addition of cod-liver oil, produced the same chemical changes (deficiency in Ca, Mg and P) in the bones of the mother and puppies, but the maternal blood Ca did not fall so much and she did not develop tetany. Instead of rickets, the young showed stiffness and soreness of the hind legs, caused by osteoporosis and multiple fractures.

An interesting observation relating to Ca and P metabolism in pregnancy is the increase in the phosphatase content of the blood which takes place. (Ramsay, Thierens and Magee, 1938; Bodansky, Campbell and Ball, 1939.) Phosphatase liberates inorganic from organic phosphate, or, working in the reverse direction, enables organic phosphate to be made. It probably plays a large part in ossification, and its increase in pregnancy may reflect the immediate demands of the foetus.

Reviewing the control of calcium metabolism in pregnancy, Bodansky and Duff (1939) question the possibility of correlating the fall in serum calcium with either the dilution or the diminished protein content of the plasma. The absence of a rise in serum-phosphorus makes it difficult to correlate with other forms of calcium deficiency. Although they attribute values below 8 mg. Ca per 100 cc. serum to dietary insufficiency, they find no evidence that the latter plays an essential part in the normal drop towards the end of pregnancy. On the other hand, they cite the following evidence for increased parathyroid activity, (1) the serum of women has a parathyroid-like action during pregnancy, (2) pregnant rats fail to support a degree of parathyroid deficiency which non-pregnant rats are able to support, (3) the parathyroids of rats are enlarged during pregnancy. Their general conclusion is that there is a readjustment of the homeostatic mechanisms of the mother, and they discuss the possibility that the foetal placenta produces a Ca-depressing factor in the mother and a Ca-elevating factor in the foetus (serum Ca is 1–3 mg. per 100 cc. higher in foetal than maternal blood, and it is unlikely that either plasma protein, which is low, or foetal parathyroid, which is probably in abeyance, are responsible).

Iron. With regard to iron, the existence of stores of widely varying capacity in non-pregnant individuals makes the interpretation of balance experiments

difficult (McCance, Widdowson and Verdon-Roe, 1938). Nevertheless, in 23 balance studies made on iron, Coons (1932) found that the average cumulative maternal storages from the 3rd to the 10th lunar months were respectively 57·4, 192·4, 340·2, 447·4, 565·0, 681·0, 754·3 and 779·1 mg. of iron. Analyses of foetal and newborn infants showed from the 5th to the 10th months respectively the presence of 10·3, 25·4, 39·5, 227·6 and 246·2 mg. of iron. (This method of

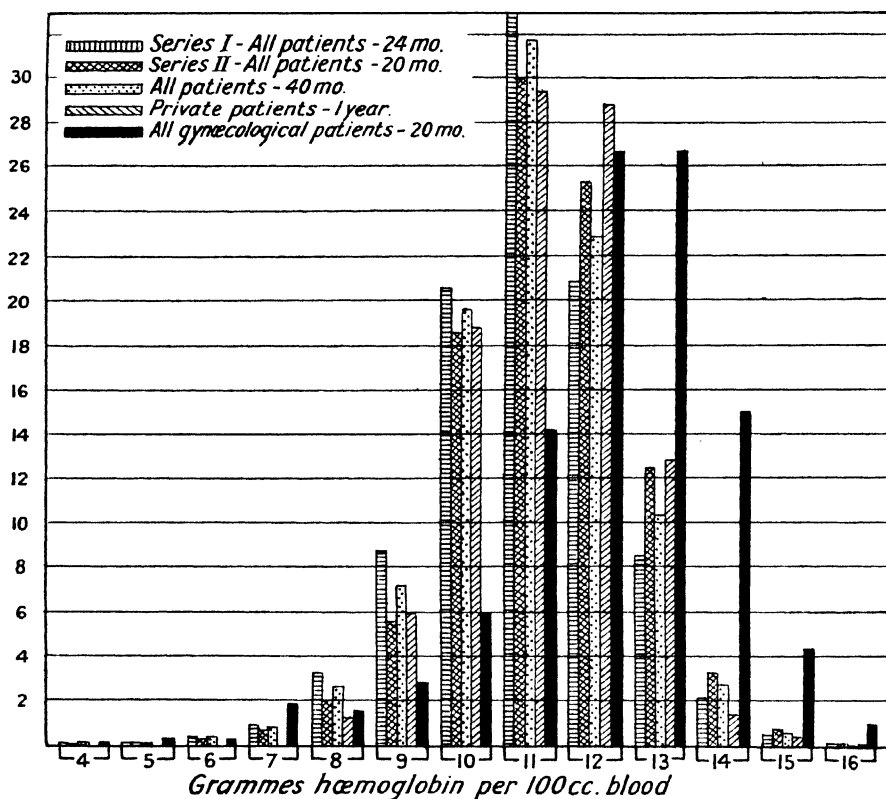


Fig. 18. 15—The frequency distribution of concentrations of haemoglobin in blood in four series of pregnant patients (shaded or stippled column), and in non-pregnant patients (black columns). (From Adair, Dieckmann and Grant, 1936.)

presenting Coons' figures is taken from Macy and Hunscher, 1934.) Although the average daily foetal needs during the 9th and 10th months exceed the average daily maternal intake, the figures indicate a maternal storage of iron greater than the needs of the foetus by more than 500 mg. According to Coons this balance is almost completely accounted for by the hypertrophied maternal muscles and other tissues.

We have referred several times to the increased blood volume which occurs during pregnancy and also to the fact that the plasma volume is increased more than that of the cells. Adair, Dieckmann and Grant refer to this as a "physiological

anaemia" and state that the mean for the haemoglobin concentration of pregnancy is 11.56 g. per 100 cc. blood, as against 14.3 g. in a non-pregnant individual. They regard the minimum standard as 10 g. of haemoglobin per 100 cc. Even then, 11½ per cent. of their patients are anaemic. Fig. 18. 15 shows the marked difference in the haemoglobin content of the blood of their pregnant patients and those of non-pregnant patients who may, nevertheless, be suffering from slight blood loss owing to gynaecological lesions. The anaemia of pregnancy is generally held to be dietary in origin but in some cases due rather to a deficiency in the utilisation of iron rather than to lack of iron itself in the food.

The discovery of Whipple and Robscheit-Robbins (1942), that post-mortem samples of liver from pregnant women were haemopoietically subnormal when assayed on standard anaemic dogs, may reflect a certain lack of ability of the pregnant organism to use iron, or perhaps merely the depletion, through excessive activity, of a haemopoietic factor. We shall see later that there is some evidence of increased red-cell breakdown and formation in pregnancy. Certain quantitative facts relating to the "anaemia of pregnancy" are critically reviewed by Fullerton (1936), and he is sceptical, with apparently good reason, of the power of pregnancy *per se* to render the mother anaemic. He considers most of the hypochromic anaemia of pregnancy to occur in already anaemic patients.

Balfour, Hahn, Bale, Pommerenke and Whipple (1942) have administered radio-active iron (Fe^{50}) to patients with a wide variety of complaints, subsequently measuring the amount appearing as haemoglobin in the blood stream. In those anaemias in which large stores of iron already exist, absorption is poor, and the authors believe that the uptake of iron by the gastro-intestinal mucosa is in some way controlled by the iron depots. Elimination of iron is a difficult matter for the body, and their experiments on dogs have established the importance of the control by intake. They state that the stores of iron are usually low during pregnancy and find the intake to be 2 to 10 times the normal.

Macy and Hunscher (1934) suggest the following nutritive requirements for human pregnancy: 70 to 100 g. of protein, 1.6 g. of calcium, 2 g. of phosphorus, 0.3 g. magnesium, 20 mg. of iron (*see also* McCance, Widdowson and Verdon-Roe, 1938).

The acid base balance.

In an attempt to satisfy themselves as to whether there is or is not a change in the pH of blood during pregnancy, Nice, Mull, Muntwyler and Myers (1936) studied 33 normal pregnant women from the 2nd or 3rd month of pregnancy to the 1st and 2nd month post-partum. Owing to doubts cast on the colorimetric method of pH determination, they measured the pH electrometrically and at the same time determined the HCO_3 , Cl and protein content of the serum (*see* Table VI). They found little progressive change during the period of pregnancy covered by their observations, dilution of the blood having presumably already taken place, but by comparing the pre-partum with post-partum values they concluded that there had been a slight fall in the alkali reserve of the blood, but no change in pH. Since the pH of the blood is dependent on the constancy of the ratio between dissolved and combined CO_2 it follows that the plasma should contain less of the former and therefore that the carbon dioxide tension of the alveolar air should be reduced.

TABLE
 NICE *et al.*, 1936)

NT AL ERAGES OF THE AC B EQU BRUN THROUGHOUT PARTUM AND Po PARTUM PERIODS

	No	pH _e *	pH	-HCC m. Eq	Cl Eq	Protein m. Eq.	Total Acid m. Eq.	Total Base m. Eq.	Undetn. Acid m. Eq.
<i>Ante-partum</i>									
8 months	4	7.42	7.47	22.7	101.1	14.0	137.8	142.7	4.9
6 and month.	23	7.39	7.39	23.3	102.2	14.4	139.8	145.5	5.7
4 and month.	26	7.41	7.425	23.2	103.5	14.5	141.2	148.1	6.9
2 and month.	28	7.41	7.42	23.7	102.8	14.3	140.3	145.7	5.4
1 mon 1	23	7.41	7.40	23.3	101.7	14.7	139.7	147.0	7.3
2 days .	9	7.42	7.445	22.1	104.0	14.9	141.0	146.1	5.1
In labour .	13	7.395	7.41	22.1	102.7	14.9	139.7	149.1	9.4
<i>Post-partum</i>									
3 days	11	7.41	7.40	24.7	103.5	14.7	142.9	150.2	7.3
7 days	21	7.41	7.40	25.0	102.2	16.1	143.3	149.7	6.4
1 mon	19	7.41	7.40	25.3	101.9	15.5	142.7	150.6	7.9
2 to 7 mon	14	7.41	7.41	26.6	102.6	15.8	145.0	149.0	4.0
Me partum		7.411	7.426	23.1	102.8	14.4	140.3	147.1	6.8
M		7.416	7.403	25.3	102.6	15.6	143.6	150.0	6.4
D		0.005	0.0253	2.2	0.24	1.16	3.3	2.9	—
Error of Differ		0.0036	0.0055	0.172	0.319	0.169	0.357	0.485	—
Ratio D/E		1.5	0.5	12.7	0.75	6.8	9.2	6.0	—

pH_e stands for electrometric pH, and pH_c for colorimetric pH.

TABLE VII

A COMPARISON OF THE ANTE-PARTUM AND POST-PARTUM CHANGES OF THE ACID-BASE BALANCE OF THE BLOOD IN NORMAL PREGNANCY
(Nice *et al.*, 1936)

	No. of Observa- tions.*	pH	HCO ₃ m. Eq.	Cl m. Eq.	Protein m. Eq.	Total base m. Eq.	pH method employed.	Author and date.
Ante-partum	55	7.44	20.5	107.6	—	—	Colorimetric	Gaebler and Rosene, 1928 (<i>Amer. J. Obstet. & Gynec.</i> , 15, 808).
Post-partum	30	7.41	24.2	107.8	—	—		
Ante-partum	12	—	22.1	103.7	15.2	146.1	Electrometric	Oard and Peters, 1929 (<i>J. Biol. Chem.</i> , 81, 9).
Post-partum	3	—	26.0	102.6	17.2	151.2		
Ante-partum	3	7.37	20.7	104.0	14.1	147.0	Electrometric	Stander, <i>et al.</i> , 1930 (<i>J. Biol. Chem.</i> , 85, 233).
Post-partum	4	7.34	25.4	106.6	16.4	155.3		
Ante-partum	100	7.39	22.1	103.4	—	152.6	Colorimetric	Dieckmann and Wegner, 1934f.
Post-partum	80	7.39	22.5	103.1	—	153.3		
Ante-partum	120	7.41	22.1	102.8	14.4	147.1	Electrometric	Present work.
Post-partum	65	7.42	25.3	102.6	15.6	150.0		

In a number of instances the data have been recalculated to terms of m. Eq. The figures given in the table are means, except for data of Oard and Peters, and Stander *et al.*, where averages are used.

* Maximum number of observations.

A reduction to between 30 and 35 mm. Hg was, in fact, found by Hasselbalch and Gammeltoft (1915), having been first noticed by Hasselbalch in 1912, and has been fully confirmed since by numerous observers. Reference to the reviews by Harding (1925), Stander (1936), and Dieckmann and Wegner (1934e) reveals that for many years there has been general agreement that a "compensated acidosis" or "compensated alkali deficit" of the kind we have described occurs during pregnancy (Table VII). We have quoted the results of Nice *et al.* because, having followed their subjects throughout pregnancy, they are able to confirm the observation of Hasselbalch and Gammeltoft that the changes occur very early. Table VIII is taken from a thesis by Hinterlang (1937), of which only an abstract

TABLE VIII
(Hinterlang, 1937)

Condition of Subject.	Vol. % CO ₂ .	
	Mean.	Range.
I. Normal non-pregnant	54·9	51·5-60·6
II. Preg. Months 2-5	51·3	46·7-53·8
" " 6	49·8	48·3-51·4
" " 7	47·8	41·8-51·9
" " 8	48·9	45·9-50·5
" " 9	48·2	45·9-50·5
" " 10	48·1	42·8-53·7
III. During labour	42·0	37·0-45·3
IV. 1-5 days post-partum	50·9	44·8-55·3

is available, but he also appears to have followed a number (not specified) of cases throughout pregnancy, and his results, given in terms of total CO₂ in 100 c.c. of blood, again indicate the early onset of the change. We are unaware of any satisfactory explanation of either the diminution of alkali reserve or the time of its appearance. If acid metabolites were the primary cause, using up the alkali reserve, then in order to reduce the dissolved CO₂ of the plasma to a corresponding degree, and so maintain the pH constant, hyperventilation would be necessary to effect the requisite fall in alveolar CO₂ pressure. The mere occurrence of hyperventilation during pregnancy is, therefore, no proof that hyperventilation is itself the essential factor. Nevertheless, such a state of affairs would be equally in accord with the observed facts. A "washing-out" of CO₂ from the blood on account of some unaccustomed stimulus to respiration might cause a compensatory excretion of alkali by the kidney.

Carbohydrate and fat metabolism.

It is unlikely that the manner in which the pregnant organism deals with fats and carbohydrates will be fully understood until the endocrine readjustment underlying pregnancy is finally elucidated. If we neglect the vitamins for which fat is the vehicle, these two classes of foodstuff raise no problem of specific insufficiency; so far as we know, their function is to supply energy and building

material, the latter of a non-specific kind. The animal body has the power of manufacturing fat from carbohydrate, and the only obvious requirements of pregnancy would appear to be that sufficient of the latter should be available. This is as much as to say that the pregnant organism should not be subjected to the risk of starvation, and raises no problems peculiar to pregnancy. The provision of sufficient carbohydrate to keep first the placenta and then the foetal liver charged with glycogen is a minor quantitative problem.

There are, however, indications that while this point of view may be legitimate from the purely dietary standpoint, there is some modification of the metabolic fate of these substances during pregnancy.

Carbohydrate. Glycosuria occurs frequently in human pregnancy, but as it may come and go in the same individual, estimates of its incidence vary widely. Rowe, Gallivan and Matthews (1931) found sugar in the urine at one time or another in 35 per cent. of their cases though only 18 per cent. showed it repeatedly. Reviewing the subject in 1925, Harding concluded that the blood sugar in pregnancy was within normal limits, that pregnancy did not cause any characteristic deviation within those limits and that administration of 100 g. of glucose caused the usual rise and fall in the blood sugar level. The last test, however, gave rise so frequently to glycosuria that it was proposed as a test for pregnancy. Rowe and his colleagues agree that the blood sugar level is within normal limits during pregnancy, but their very careful work in which the normality of their subjects "was a matter of objective investigation and not of assumption" shows that the level is consistently in the lower range of the normal. The pre-partum average (covering the greater part of pregnancy) was 83 mg. per 100 cc. of blood and the post-partum average 94 mg.

It seems certain, therefore, that the glycosuria of pregnancy cannot be attributed to a high blood sugar level, and it is generally assumed that the renal threshold for glucose is low.

The value of blood sugar tolerance curves is difficult to assess in view of the importance of having the diets of the subjects properly controlled. Harding qualifies his statement regarding the normality of such curves with a report by one observer (Bauer) that unusually high values were found after the ingestion of glucose. This finds an echo in the more recent work of Tonkes (1940) who, in a short series of nine normal women at unspecified stages of pregnancy, describes a wide scatter in the peak value of blood sugar curves following 50 g. of glucose. Mandruzzato (1937, quoted from an abstract) gave his subjects two successive doses of glucose and found normal responses in normal pregnancy. In toxæmia, however, he found an abnormal increase in blood sugar after each dose. It would seem wise to assume, at the moment, that instability in the response to a glucose tolerance test may be a sign of an incipient abnormality rather than a normal consequence of pregnancy, or alternatively, that if such instability is normal it operates equally in either direction. The curve drawn by Tonkes from the average of all his readings is indistinguishable from a normal response.

The course of diabetes is known to be modified by pregnancy but not, so far as one can tell, in any consistent way. In this connection we cannot do better than quote the pungent summing up of the situation by Cuthbert, Ivy, Isaacs and Gray (1936), "The literature on the effect of pregnancy on the severity of the diabetic condition is discordant

“Carlson and his collaborators observed that hyperglycaemia and glycosuria did not result or was (*sic*) slight following pancreatectomy in gravid dogs near term. This was confirmed on one dog each by two observers (Lafon, and Simon, Stulz and Aron). On the contrary, Allen failed to observe that pregnancy had an effect on partially depancreatized dogs, and Markowitz and Simpson observed no change in carbohydrate metabolism during pregnancy in a depancreatized dog (inadequately studied) receiving adequate doses of insulin. However, the latter observers reported the occurrence of hypoglycemic symptoms after parturition in their dog, which was attributed to the formation of milk. Later, Markowitz and Soskin studied another depancreatized pregnant dog, which received insulin in doses just inadequate to prevent glycosuria, and observed no significant change in sugar excretion as pregnancy advanced. They also repeated the work of Carlson on two dogs, with the exception that they administered insulin in small (3-5 u. daily) doses, and failed to obtain evidence confirmatory of Carlson's results.

“A review of the post-insulin clinical literature on this subject reveals that some report that pregnancy intensifies and others that it ameliorates the diabetes. Most agree that the state of early pregnancy intensifies or does not change the diabetic condition and that lactation ameliorates it. Kramer recently on reviewing one hundred and ten cases in the literature, found that in late pregnancy the sugar tolerance was impaired in 29 per cent., unchanged in 24 and improved in 47 per cent. of patients. Hypertrophic and hyperplastic islets have been observed in several infants born of diabetic mothers and hypoglycemic symptoms in such infants have also been observed.”

In an attempt to resolve some of these difficulties experimentally Ivy and his colleagues discovered a very interesting fact. In accordance with the experimental findings of others and with the majority of clinical opinion, they found that pancreatectomy performed on the pregnant dog near term did not give rise to diabetes if the number of foetuses was more than two. In the absence of a method for measuring the amount of insulin in the blood they refrained from drawing the obvious conclusion that the foetal islets were responsible. In order to stimulate more closely the conditions found clinically they depancreatized dogs before pregnancy and maintained them on a uniform diet with a dose of insulin which permitted a small degree of glycosuria. During the early part of pregnancy glycosuria was increased but was later diminished and the insulin requirement decreased. The improvement in late pregnancy was by no means comparable in degree to the resistance of dogs to acute pancreatectomy late in pregnancy, since it reduced the insulin requirement only by about 25 per cent. The authors regard utilisation of carbohydrate by the foetuses as a more likely explanation of this slight improvement than the production of large quantities of foetal insulin, because the improvement is maintained and even enhanced during lactation. They are unable to suggest an explanation for the difference between the chronically and acutely depancreatized animals. The results obtained with the former, in which the diabetic condition is at times improved and at times made worse, resemble the clinical findings.

Lactose appears in the urine in a large number of pregnancies shortly before delivery and in the majority afterwards. It may be regarded as due to overproduction (Rowe, Gallivan and Matthews, 1936).

Rowe and his colleagues have made interesting observations on the tolerance of galactose, a test for liver function based on the ability of the normal liver to synthesise glycogen from this sugar. (In impaired liver function, galactose is excreted in the urine.) The depression of the tolerance during pregnancy, and particularly during lactation (Fig. 18. 16), may simply be an expression of the fact that one of the functions of mammary tissue is to synthesise galactose, and the liver may, therefore, be dealing with greater quantities of this substance than are involved in the test dose.

Fat. Most of the investigations of fat metabolism in pregnancy have been made with the object of elucidating the mechanism by which the foetus gains the fat it requires for essential tissue-building purposes. This problem is discussed elsewhere in this book and concerns chiefly the function of the placenta. There is, however, a well known and very definite maternal reaction in women, the cause

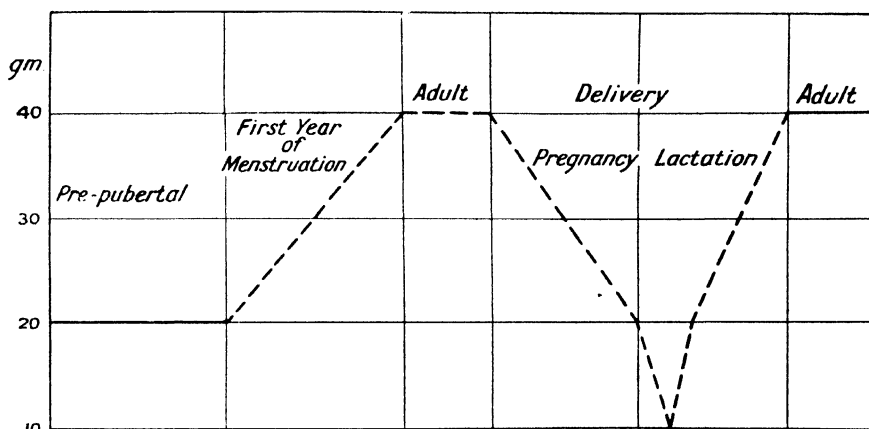


Fig. 18. 16—Influence of sexual status on tolerance level of galactose.
(From Rowe, Gallivan and Matthews, 1931.)

of which has not been found. There is a marked increase in the quantity of blood lipids, including cholesterol, neutral fat and phospho-lipids. The increase is almost entirely in the plasma, the content of the corpuscles being relatively unchanged, and it varies from 25 per cent. to 50 per cent. in the different lipid fractions (Boyd, 1934). Schwarz, Soul, Dunie (1940) have confirmed the results of earlier observers, whom they quote in their bibliography, and have contributed records of eight individuals whose blood lipids they have followed throughout pregnancy. As in many of the changes associated with pregnancy this type of study is important because individual variation is so great that averages taken from groups of subjects are apt to be unconvincing. Both the individual variation and the steady upward trend of total lipids is shown in Fig. 18. 17 in which each curve is a record of a single subject. Percentage increases for the fractions varied from 44 to 172 per cent. for neutral fat, from 30 to 117 per cent. for total fatty acids, from 8 to 136 per cent. for total cholesterol, from 4 to 150 per cent. for ester cholesterol, from 20 to 113 per cent. for free cholesterol and from 20 to 160 per cent. for phospho-lipid.

Whether this hyperlipaemia has anything to do with the transfer of fat to the foetus is difficult to say. It does not seem to imply difficulty on the part of the liver in dealing with fat, for Guercio and Riboni (1937a) and Manzi (1936) did not find any conspicuous abnormality after a "test meal" of fat during pregnancy. Guercio and Riboni (1937b) found that if 100 g. of glucose was added to the test meal, only the concentration of phospho-lipids rose in the serum, that of fat and cholesterol falling. Mühlbock (1937) finds the average iodine value of the serum increases during pregnancy from the fourth month onwards reaching its highest level just before delivery and maintaining it for a further week.

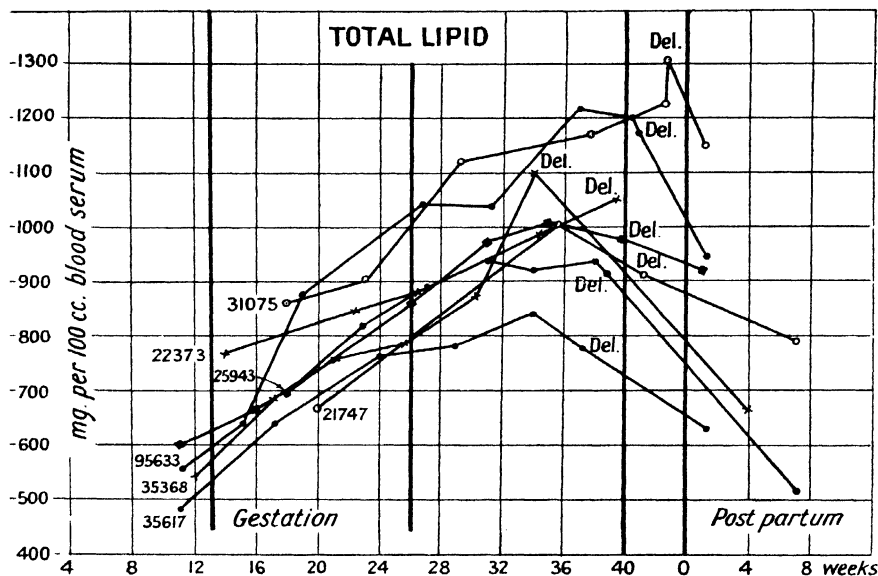


Fig. 18. 17—The concentration of total blood lipid measured in 8 women at intervals throughout pregnancy. (From Schwartz, Soul and Dunie, 1940.)

Although it is generally taken for granted that there is a tendency to ketonaemia and ketonuria during pregnancy, Gray (1938) found normal blood ketone values in 3000 pregnant women. It seems, however, well established that in starvation or insufficient carbohydrate intake, ketonuria may rapidly supervene.

Vitamins.

An enormous literature exists on the requirements of pregnant women for the various vitamins. It is doubtful, however, whether there is anything unusual about the metabolism and functions of the vitamins during pregnancy. It is generally assumed on fairly good evidence that the pregnant woman requires an assured and adequate supply of these accessory foodstuffs, and that the consequences of deficiency may be more serious for her and her offspring than for a non-pregnant individual. The ground is very well covered in the review by Garry and Stivin (1936).

V. THE BLOOD

The most important changes in the blood have already been dealt with under other headings. It is natural that this should be so, and that the changes we regard as proper to blood itself should appear as apparently isolated phenomena.

Sedimentation rate.

The rate at which the corpuscles sediment in blood plasma is greater than that at which a single corpuscle should sink, in proportion to the tendency of the corpuscles to form rouleaux, i.e. to increase the ratio of mass to surface in a corpuscular particle. Clumps of 11 corpuscles, for instance, sink at a rate of about 1 mm. per hour, and of 58,000 corpuscles at about 75 mm. per hour. The average normal rate for men is about 3 mm., for non-pregnant women about 7 mm. and for pregnant women at term about 40 mm. per hour. (Table IX.) The rate is,

TABLE IX
AVERAGE SEDIMENTATION TIMES IN 100 PREGNANT WOMEN
(Fåhræus, 1929)

Month of Pregnancy.	Mm./hr.
2	17
3	24
4	20
5	29
6	33
7	40
8	47
9	45
Month of Puerperium.	
1	41
2	20

however, very variable in the latter, as the frequency distribution curve of Fig. 18. 18 indicates (Bager, 1936).

The phenomenon was first described by Fåhræus in 1918 and further information on the subject will be found in his reviews (1929, 1939). The tendency of red cells to agglutinate is not completely understood, but appears to depend partly on the concentration of globulin and fibrinogen in the blood plasma. Fåhræus postulates that these proteins neutralise the negative electrostatic charge which is carried by the corpuscles and which normally maintains them in a state of mutual repulsion. The higher the concentration of the proteins, the faster the sedimentation rate. In normal pregnancy, the concentration of serum globulin is slightly increased, or diminished less than that of albumin, so that the globulin-albumin ratio is increased (Fig. 18. 7). Whether this relative increase of globulin can have any effect is difficult to say. Fibrinogen, which occurs only in plasma and not in serum, is definitely increased in concentration (Dieckmann and Wegner, 1934d), though Melnick and Cowgill (1937) find that the rate of regeneration of plasma proteins by the liver in dogs is actually diminished in pregnancy, and they attribute this to the requirements of the foetus and mammary glands.

Ropes, Rossmeisl and Bauer (1939) express doubt as to an exact casual relationship between the concentration of plasma proteins and sedimentation rate, which they have studied in various diseases. The commonly accepted parallelism exists, but in group observations a large proportion of individuals do not conform, and in the same individual alterations in sedimentation rate do not consistently precede or accompany changes in either fibrinogen or globulin. These authors agree with Fåhræus that the phenomenon depends on the relation between the electrical charges of the corpuscles and colloids, but believe that other factors besides the actual concentration of the latter may intervene. It is perhaps worth remarking here that Dieckmann and Wegner (1934d) imply that the diminution in the concentration of serum albumin in pregnancy, which they find to be less than do most observers, is insufficient to account for the lowered colloid osmotic pressure of the plasma and they suggest intrinsic alterations in the proteins themselves.

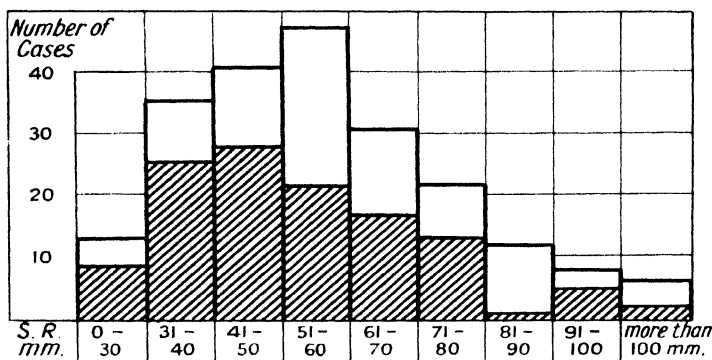


Fig. 18. 18—Normal cases arranged according to value of sedimentation reaction (S.R.) before delivery. The heavy parts of the columns denote those cases where S.R. increased during puerperium; the rest, cases where S.R. diminished. (From Bager, 1936.)

The number of white cells in a given volume of blood increases somewhat during pregnancy (*see* Lochhead & Cramer, 1922), but not greatly beyond normal limits. Carey and Litzenberg (1936) in nearly 1000 blood counts in 134 pregnancies found that in 50 per cent. the count was between 8700 and 12,500, independent of the stage of pregnancy. Daniachij (1936) has studied the sternal bone marrow in normal pregnancy and finds a marked increase, reaching a maximum of 6 times the normal by the 8th month, in the neutrophil myelocytes (precursors of the polymorphonuclear leucocytes).

According to Possaghi (1937) the increased activity of the bone marrow extends to the formation of red cells. He found the average concentration of reticulocytes in the blood to be approximately doubled. (Normal controls, 0.59 per cent.; 2nd month of pregnancy 0.92 per cent., gradually increasing to 1.2 per cent. in the first week of the puerperium.) The scatter of individual values is large, but a superficial examination of the protocols suggests that the averages may indicate a genuine trend. All these observations have been confined qualitatively, if not strictly quantitatively, in an extensive investigation by Pitts and Packham (1939). The results of their examination of the sternal bone marrow are summarised in Table X.

ABLE X
 RANGE AND RANGE OF V FOR STERNAL MARROW OF PREGNANT AND OF NON-PREGNANT WOMEN AND PACKHAM
 1939 COMPARED WITH THE NORMAL RANGE FOR STERNAL MARROW

	Normal (Male).			Non-pregnant women.			Pregnant women.		
	Range per c. mm.	Average per c. mm.	Range per c. mm.	Range per c. mm.	Average per c. mm.	Range per c. mm.	Average per c. mm.	Range per c. mm.	Average per c. mm.
Total nucleated cell count.	6,000 to 60,000 per c. mm.		7,750 to 46,000 per c. mm.		23,100	14,400 to 125,000 per c. mm.		36,760	
Myeloblasts	0-0-2-0	0-44	0-0-0-8	0-0	0-0	0-0-1-0	0-0	0-24	0-0
Promyelocytes, type A	0-0-5-0	1-48	0-1-2-2	0-1-2-2	1-0	0-0-2-3	0-0-2-3	0-93	0-82
Myelocytes, type S	0-0-5-0	1-68	0-0-1-4	0-0-1-4	0-5	0-0-2-8	0-0-2-8	0-40	6-25
Eosinophilic myelocytes	0-0-2-0	0-64	0-0-1-5	0-0-1-5	0-5	0-0-2-6	0-0-2-6	5-46	
Neutrophilic myelocytes	0-0-10-0	0-86	1-4-11-0	1-4-11-0	6-0	1-2-12-5	1-2-12-5		
Neutrophilic metamyelocytes	1-0-10-0	7-4	1-4-9-4	1-4-9-4	5-6	1-6-9-0	1-6-9-0		
Unsegmented polymorphonuclears	15-0-35-0	24-1	12-4-34-2	12-4-34-2	23-7	15-2-47-6	15-2-47-6	33-3	
Segmented polymorphonuclears	7-0-25-0	13-3	10-4-31-0	10-4-31-0	16-6	1-6-37-6	1-6-37-6	15-7	
Eosinophils	0-0-2-6	0-8	0-2-6-0	0-2-6-0	1-3	0-0-6-0	0-0-6-0	0-88	
Basophils	0-0-0-2	0-1	0-0-0-8	0-0-0-8	0-3	0-0-1-0	0-0-1-0	0-2	
Lymphocytes	4-0-16-0	10-6	9-2-30-4	9-2-30-4	16-8	4-5-28-0	4-5-28-0	12-7	
Monocytes	0-0-5-0	2-06	0-0-3-5	0-0-3-5	1-4	0-0-3-0	0-0-3-0	0-77	
Karyoblasts	0-0-0-2	—	0-0-0-4	0-0-0-4	0-1	0-0-1-0	0-0-1-0	0-17	
Prokaryocytes	0-0-5-0	1-5	0-6-4-0	0-6-4-0	2-5	0-3-6-2	0-3-6-2	2-37	
Karyocytes	2-0-15-0	7-0	1-3-8-0	1-3-8-0	5-0	0-3-13-6	0-3-13-6	4-3	
Metakaryocytes	2-0-10-0	5-0	0-4-3-2	0-4-3-2	1-9	0-2-7-0	0-2-7-0	2-26	
Disintegrated cells	12-8-31-8	20-8	10-5-24-0	10-5-24-0	16-2	5-5-23-6	5-5-23-6	12-93	

Possaghi links up the increase in reticulocytes with the following indications of increased red-cell destruction which are said to occur during pregnancy. (1) enlargement of the spleen, (2) a high incidence of haemoglobinaemia, (3) a 30 per cent. increase in the urobilinogen content of the urine, (4) a deposit of haemosiderin in the placenta.

Fåhræus also quotes enlargement of the spleen in human pregnancy, and in his view the packing of red cells in this organ is a device for reducing the cell-plasma interface. The extensive rouleaux formation associated with the high sedimentation rate must reduce the total interface also in the circulating blood. If, as Fåhræus appears to believe, the two phenomena represent an integrated reaction, the question of its consequences arises. One of these is to render the erythrocytes more liable to haemolysis which, though in accordance with the increased blood destruction stated by Possaghi and Fåhræus to occur in pregnancy, is of questionable value to the organism. It may, however, be a method of making iron available to the foetus. A difficulty in this idea of Fåhræus is that the reduction of interface leads to the production of substances in the plasma which diminish the tendency of the erythrocytes to aggregate. In addition, the red cells themselves spontaneously lose this tendency when separated from plasma. It seems necessary to postulate an equilibrium between the cell-packing effects of the plasma proteins and spleen on the one hand, and the cell-scattering effects of the resultant changes in the corpuscles and plasma, in which the first effect predominates.

Although this conception is not altogether satisfying, it is provocative because Fåhræus finds that lysolecithin is probably responsible for the cell-scattering and haemolytic consequences of a reduction in the plasma-cell interface, and he believes this substance could be accounted for by the retardation of certain stages of fat metabolism in which the red cell surface might play a part. "Concerning the cause of lipaemia in pregnancy there are, as Boyd (1934) pointed out, no less than fifteen different theories. Herewith I put forward the sixteenth: that the alteration of the fat metabolism of the blood during pregnancy is due to a decrease of the erythrocytes-plasma interface, which is very probably a significant manifestation of the decreased suspension stability of the blood." (Fåhræus, 1939.)

A hydrodynamical result of the aggregation of erythrocytes will be to reverse the position of red and white cells in the small blood vessels, so that the latter will take up a peripheral position and travel more slowly. They will, therefore, tend to exist in higher concentration in such situations, and it is possible that the raised leucocyte count as determined by the usual finger-prick method may be partly accounted for on these grounds.

VI. THE KIDNEYS

Since the kidneys are involved in the toxæmia of pregnancy, the assessment of renal function must be made on cases in which pregnancy fulfils the other criteria of normality. The high incidence of toxæmia is an indication that kidney function is apt to become abnormal, but we are concerned only with normal pregnancy.

A great deal can now be learned about events in the kidney by observing the excretion of certain substances. The polysaccharide inulin is filtered by the glomerulus but completely ignored by the tubules. The quantity appearing in the urine in a given period is, therefore, a measure (if its plasma concentration is known) of the volume of deproteinised plasma which has been filtered off by the glomerulus.

The substance diodrast, in addition to being filtered off by the glomerulus in the usual way, is also secreted so actively by the tubules that the blood emerging from the kidneys is completely freed from it. The amount appearing in the urine in a given time can, therefore, be used as a measure of the blood-flow through the kidney during that time. Other substances, like phenol red, are secreted by the tubules, but less readily, so that the contribution made by the tubules to excretion bears some relation to their functional activity.

Welsh, Wellen, Taylor and Rosenthal (1942) find that normal pregnancy does not alter the filtration rate, the effective renal blood flow or the " tubular excretory mass " (an index of tubular activity). Chesley and Chesley (1939) also find the renal blood flow normal. Brown (1938) has determined the urea clearance in a number of normal pregnant women and found it somewhat greater than normal, i.e. the kidneys remove urea from the blood stream rather more effectively than usual. In view of these findings which were adequately controlled by results on normal non-pregnant subjects, it would appear that there is no gross interference with renal function during normal pregnancy.

The abnormality in toxæmia of pregnancy seems to be a reduced filtration rate, i.e. the volume of blood passing through the kidney is normal, but the quantity of plasma filtered through the glomerulus is diminished. The urea clearance is also diminished roughly proportionally to the severity of the disease. The deficiency of renal function in toxæmia has long been known and has given rise to such speculations as to whether it could be due to interference by pressure with the circulation in that organ. Dill and Erickson (1938, 1941), Dill, Eisenhour and Cadden (1939) finds that renal ischaemia produced in animals by means of constricting wire loops on the renal artery ultimately causes death, and that pregnant animals are more susceptible than non-pregnant. The experimental syndrome is said to resemble that of toxæmia, but apart from the fact that the pregnant animal succumbs earlier, thereby implying a greater dependence on the function of the kidney, the results reveal no feature of especial interest.

Another way in which the excretion of urine could be interfered with is by the production of a chemical substance resembling the posterior pituitary principle. The appearance of such substances has been reported by various observers. For instance, Teel and Reid (1939) find that one may appear in the urine in the last three months of a normal pregnancy under conditions of dehydration but not otherwise. It is, however, readily demonstrable in the urine of eclamptic patients. Krieger and Kilvington (1940) find such a substance in urine concentrates from some non-pregnant and, somewhat more frequently, pregnant women. The urine of eclamptic patients appears to be rich also in this substance. Griffith, Kimbrough, Corbit and Roberts (1942) claim that pregnancy serum often contains an anti-diuretic substance. Once again the balance of evidence seems to reveal little of interest in normal pregnancy.

VII. RESPIRATION AND CIRCULATION

The respiratory and the circulatory systems are of interest in pregnancy only in so far as they are considered together in relation to the oxygen supply of the foetus. Numerous observations have been made on variables like the vital capacity in human pregnancy for clinical reasons, but they reveal very little alteration, and the most striking modification so far as the respiratory apparatus is concerned has already been mentioned, namely, the lowered tension of alveolar carbon dioxide. The shape of the thorax is obviously altered in human pregnancy, the costal angle becoming wider and respiration tending to become more "thoracic" in nature. None of these mechanical modifications seems of great fundamental importance. The dyspnoea and lowered capacity for physical work associated with the later months of gestation are a circulatory rather than a purely respiratory problem. Conversely, the importance of the circulatory adjustments is largely connected with the supply of oxygen to the tissues, and is to that extent respiratory in nature.

Although the maternal heart is spared the actual work of pumping blood round the foetal circulation, and perhaps, in consequence, participation in some complicated cardio-vascular reactions, it still has to supply the oxygen and fuel for the foetal heart to carry out this task. The foetal circulation picks up its oxygen from the blood of the maternal sinuses, and the necessity for a liberal supply of blood to bathe the placental villi, in order that the "step down" between maternal and foetal circulations shall not be too great, is likely to make appreciable demands on the maternal heart. It may be, for instance, that more maternal blood passes through the uterus in a given time in order to maintain this arrangement than would be required to supply the foetus at first hand, though its passage is much easier. The work of Burwell and his collaborators has led them to the conclusion that the uterine circulation during pregnancy acts like an arterio-venous fistula. So far as we know this point of view has not been seriously disputed and it serves so well to co-ordinate the otherwise isolated facts which have been observed with regard to the circulation in pregnancy that we may be justified in adopting it. For the full literature on the subject four fairly recent reviews may be consulted, namely, those by Schroeder (1932), Burwell, Strayhorn, Flickinger, Corlette, Bowerman and Kennedy (1938), Jensen (1938), and Cohen and Thomson (1939).

Burwell bases his conception of the placenta as a modified arterio-venous fistula on the following findings :—

(1) The cardiac output per minute is increased. This is in accordance with the findings of most other workers from Lindhard (1915) onwards, including Stander, Duncan and Sisson (1926) who used pregnant bitches. The outputs in two dogs were respectively 3073 cc. and 2467 cc. when not pregnant, and 4165 cc. and 3781 cc. when pregnant. The increase was, therefore, between 25 and 33 per cent. of the original output. The values for women vary a good deal but appear to be of about the same order. The average changes in 4 women studied continuously by Burwell and his colleagues are shown in Fig. 18. 19, and it will be noticed that the values are well above normal although showing a downward trend in the last month of pregnancy.

(2) The cardiac output seems to increase faster than the oxygen consumption, which was measured regularly in the same 4 women. This implies that the blood returned to the right heart should become more arterial and it was actually found to do so up to the 36th week of pregnancy. After that, no doubt as a result of diminishing cardiac output and the greater oxygen usage by the foetus, the arterio-venous oxygen difference was increased.

(3) As we have seen, the venous pressure in the legs is raised while that in the arms remains constant. Burwell found that the pressure in the femoral vein of pregnant dogs was greater than that in the jugular vein and was not lowered by opening the abdomen. In order to achieve a comparable femoral venous pressure by increasing the intra-abdominal pressure, the wall of the abdomen had to be rendered tense by the introduction of a considerable quantity of saline.

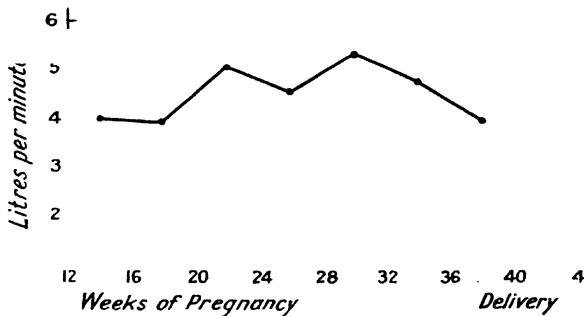


Fig. 18, 19—The cardiac output in the fourth to the tenth month of pregnancy and in the puerperium. (From Burwell *et al.*, 1938.)

In view of the previous observation, and as the abdominal wall of pregnant women is not tense, Burwell is not inclined to regard increased intra-abdominal pressure as causing the obstruction to venous return from the legs. He concedes that it may be partly due to direct pressure of the uterus on the great veins, but in the pregnant bitches it was found that when such direct pressure was eliminated by supporting the uterus independently, the femoral pressure, though less than before, still remained above normal. Pressure in the uterine veins, when measured, were even higher than those in the femoral, and surgical removal of the uterus restored the femoral venous pressure to the same level as the jugular. The numerical results suggest that these progressive experiments in dogs require confirmation in a larger series of animals. Meanwhile, there is no doubt that the conclusion that increased venous pressure in the legs is partly due to a large outflow of blood from the uterus is a reasonable supposition.

(4) There is a progressive increase in pulse rate.

(5) There is a tendency for the pulse pressure to rise.

(6) The blood volume increases.

All these changes are also characteristic of arterio-venous fistulae, and Table XI certainly shows a remarkable parallelism between the general circulatory changes of this condition and those of pregnancy, though it is obvious that these criteria alone are scarcely sufficient to establish the identity of the two conditions. The observations do, however, reinforce the deductions which may be made from

TABLE XI
(Burwell, 1938)

COMPARISON OF THE CHANGES IN THE CIRCULATION IN PREGNANCY AND IN ARTERIOVENOUS FISTULA

	Normal.	Arteriovenous fistula.	Pregnancy.
Continuous bruit	Absent	Present	Present
Average basal heart rate	66 to 70 per min.	82 per min.	90 per min.
Arterio-venousoxygendifference	55 to 70 c.c. per litre	38 to 46 c.c. per litre	40·5 c.c. per litre
Cardiac output per square mm. per min.	2 to 2·4 litres	3·2 litres	2·9 litres
Oxygen content of venous blood near fistula	At level of venous blood	Near level of ar- terial blood	Between levels of arterial and venous blood.
Pressure in veins near fistulous opening	Normal	High	High
Total blood volume—Females	4300 c.c.	6900 c.c.	5300 c.c.
Males	5300 c.c.		
Arterial blood pressure (mm. Hg)	110–120 70–80	102/56	112/70
Dilatation of artery proximal to leak	Absent	Present	Present

femoral venous pressure and the absence of maternal arterioles and capillaries in the placental circulation. Burwell *et al.* (1938), quote Spanner as stating that about 500 maternal arteries, with a terminal diameter of 0.15 mm. enter the intervillous spaces directly. The arteries are also said to exhibit the dilatation proximal to the point of entry which is typical of arteriovenous fistulae. The venous sinuses are, of course, interposed between the arteries and veins, and to this degree the connection is indirect. The amount of blood which can be shunted through this system is limited, as shown by the mounting desaturation of the uterine venous blood with the advance of pregnancy (Barcroft), but it probably represents the specific contribution the state of pregnancy makes to the work of the maternal heart.

It is very difficult to assess the comparative importance of this contribution when considering the total "strain" placed upon the maternal heart by pregnancy. According to Mackenzie (1921) there is no strain in the pathological sense if the heart is normal in the first place, but pregnancy clearly employs some of the adjustments normally reserved for response to muscular work and exercise, for in the later months the capacity for this type of activity is diminished. It must be remembered, however, that by the end of pregnancy a woman is carrying an additional load of, say, 24 lb., which she cannot set down and that her standing posture is modified. All this implies a considerable degree of muscular effort before any further activity is undertaken at all. The widening of the chest and the displacement of the heart probably contribute to the slight embarrassment of the pulmonary circulation described by Mackenzie, who found crepitations which disappeared on deep breathing at the lung bases of normal pregnant women after a night in bed.

Jensen has reviewed the various observations and opinions on the work of the heart during pregnancy, and it would appear that Mackenzie's estimate of the situation, much as we have outlined, is not outdated. Quantitatively, it is useful to bear in mind that the 50 per cent. increase in resting cardiac output which pregnancy may entail is, in terms of muscular exercise, about the same as that evoked by walking at 2 miles per hour.

VIII. OTHER CHANGES DURING PREGNANCY

Various other changes occur during pregnancy which do not seem to justify extended treatment in this chapter. As examples, we may mention alterations in the distribution of pigment in the skin, the excretion of histidine, dilation of the ureter, changes in the skeleton, morning sickness and other digestive disturbances. Changes in the ductless glands other than those intimately associated with reproduction probably take place, but are little understood. The thyroid enlarges in women and has been described as showing evidence of hyperplasia both in women and other animals, but there is great species variability and its presence does not appear to be essential for pregnancy.

It is doubtful how far the general picture of human pregnancy agrees with overactivity of the thyroid gland. On the one hand we have the increased metabolic rate, and on the other hand the increased blood cholesterol. A quantitative finding is that of Bokelmann and Scheringer (1931) who ascertained that the average concentration of iodine in the blood rose from 15.5 (second month) and 17.2 (third month) to 24.9 micrograms per 100 c.c. at full term. There was, however, considerable variation among individuals and the significance of the rise in concentration is, in any event, not well understood. Anselmino and Hoffman (1935a) state that the oral administration of human pregnancy serum to rats—a method which eliminates any gonadotrophic action of the serum—has three results typical of thyroid activity, namely, an increase in basal metabolic rate, a decrease in liver glycogen and an increase in blood acetone. It is evident from a discussion between Neuweiler (1935) on the one hand, and Anselmino and Hoffman (1935b) on the other that the chemical, histological, experimental and clinical evidence is in many respects equivocal, and that while some increase in thyroid activity is probable, there is no certain way of assaying it.

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CHAPTER 19

PARTURITION

By F. H. A. MARSHALL and J. CHASSAR MOIR

The innervation of the generative organs has already been briefly described in the first volume of this work. In the present chapter the nerve supply to the female generative system is dealt with in its functional aspects, and more particularly that to the uterus, since this is the organ which is especially concerned in the process of parturition. But, before passing on to the internal organs, the physiological activities of the nerves supplying the vulva and clitoris may be briefly dealt with.

I. THE NERVE SUPPLY TO THE EXTERNAL GENERATIVE ORGANS

The external generative organs in the female are similarly innervated to those of the male.

Langley and Anderson (1895) found that stimulation of the first five lumbar nerves in the cat, or the third, fourth and fifth lumbar nerves in the rabbit, produced similar effects to those in the male except that they were less pronounced. The effects were : (1) Pallor of the clitoris and of the mucous membrane of the vulva, accompanied by slight retraction of the clitoris ; (2) contraction of the vulva, and (3) contraction of the muscles of the adjoining skin, drawing the vulva dorsally towards the rectum.

Langley (1891), and subsequently Langley and Anderson (1895), found that two groups of effects, which were antagonistic to one another, could be produced by stimulating the sacral set of nerves in the vertebral canal, but that, as in the male, only those fibres which exercised an inhibitory influence ran from the spinal cord in the sacral nerve roots. The inhibitory effects produced were : (1) Flushing of the vulva and clitoris ; (2) dilatation of the vulva ; and (3) relaxation of the skin muscles surrounding the vulva. The visceral motor effects were the same as those produced by the lumbar set of nerves as described above. Besides these effects, contraction was induced in the external sphincter of the vagina and in the striated genital muscles.

II. THE NERVE SUPPLY TO THE OVARIES

The ovary is innervated from the sympathetic plexus accompanying the ovarian artery and from the plexus associated with the ovarian branch of the uterine artery. It is generally supposed that the nerve fibres, which are non-medullated, are merely vascular in function (Von Herff, 1892 ; Von Gawronsky, 1894 ; Kallius, 1895 ; Mandl, 1895), for ovulation, which in some species of animals only occurs

as a consequence of coition, is now known to result from the discharge of an anterior pituitary hormone, or some anterior pituitary-like substance. It has been suggested that the rupture is due to the stimulation of erectile tissue (Rouget, 1858).

III. THE NERVE SUPPLY TO THE UTERUS AND VAGINA AND THE MECHANISM OF UTERINE CONTRACTION

It is well known that the onset of parturition is manifested by rhythmically repeated contractions of the uterus which constitute the "labour pains." But although the contractions are most pronounced at this period, observations on animals have shown that even in a virgin uterus rhythmical movements may occur.

Kehrer (1867) showed long ago that a uterus separated from the body is capable of undergoing contractions if kept moist, and at the normal body temperature. More recently Helme (1891), Kurdinowski (1904), Franz (1904), and others have confirmed Kehrer's observation, thus proving that the movements are not dependent on impulses received from the central nervous system. These investigators showed that the excised uterus may undergo regular contractions for a prolonged period if placed in a warm bath of normal saline solution or on having its vessels perfused with Locke's solution. According to Franz the excised virgin uterus exhibits no spontaneous contractions, but Helme and Kurdinowski state that they may occur, though they are much weaker than those taking place during and after pregnancy.

The movements of the uterus have been more fully investigated by Cushny (1906), who carried out a large number of experiments upon rabbits and other animals. This author states that in virgins the unexcised uterus may remain motionless for a long time, but that after manipulation or exposure to air rhythmic contractions are often developed. He was disposed to believe, therefore, that the virgin uterus in the intact animal undergoes no spontaneous movements. In animals in a state of "heat," and during and after pregnancy, spontaneous contractions could generally be discerned from the first, and the author doubted if the organ ever resumed its original inert condition after it had once been pregnant. In some cases the movements seemed to occur almost simultaneously throughout the entire organ, but in others the circular muscle fibres contracted independently of the longitudinal, and vice versa. Mechanical or electrical stimulation caused very powerful contractions, but these were elicited more easily in the pregnant than in the virgin uterus, while the increased irritability was found to persist after pregnancy was over.

Helme stated that a shutting off of the blood-supply in the excised and perfused uterus of the sheep brought about contraction. Kurdinowski, on the other hand, found that in the intact animal the opposite effect was produced. Cushny's experiments for the most part confirm those of Kurdinowski, but clamping the aorta in the cat led to conflicting results, since in two cases it was succeeded by relaxation and in three by contraction. No reason could be assigned for this divergence.

It has long been known that uterine contractions can be induced by nervous stimulation. Feldman (1920) states that there are probably centres for uterine contraction in the cortex, medulla, and cerebellum, since stimulation of these areas causes uterine contractions, but he does not cite his authorities. Serres (1824) showed that irritation of the spinal cord in the lumbar region excited the uterus

to contract, and later investigators have obtained similar results (Budge, 1858; Riemann, 1871). Röhrig (1879) showed that asphyxia which may bring about uterine contractions (and abortion in the pregnant condition) cannot do so if the lumbar cord is destroyed. Frankenhauser (1867) and Körner (1865) discovered that the efferent nerve fibres left the lumbar region of the spinal cord, and after traversing the sympathetic, the inferior mesenteric ganglia and the aortic plexus, made their way to the uterus. Langley (1891) found that the majority passed to this organ by way of the sympathetic in the region of the fourth, fifth, and sixth lumbar ganglia, so that they probably arise from the third, fourth and fifth spinal nerves. Subsequently, Langley and Anderson (1895) showed that stimulation of the second, third, fourth and fifth lumbar nerves (in cats and rabbits) causes pallor and contraction of the Fallopian tubes, uterus, or vagina, but that stimulation of the first and sixth lumbar nerves produces no effect. They state that the efferent fibres are motor for the muscular walls and vaso-constrictor for the small arteries. The effect on the uterus and vagina was found to vary with the state of the uterus in regard to parturition. Langley and Anderson state that the sacral nerves send neither motor nor inhibitory fibres to any of the internal generative organs, thus differing from Kehrer, Körner, and others, who say that they obtained contraction of the uterus on stimulating these nerves.

Keiffer (1932) also independently investigated the innervation of the uterus, and the results obtained by exciting various nerves, his observations agreeing for the most part with those of Langley and Anderson.

Cushny, in the paper already referred to, has described at some length the effects of hypogastric stimulation, which produced in the rabbit powerful contraction of the whole uterus irrespective of its condition in regard to the occurrence of pregnancy. If the stimulation was prolonged for more than fifteen seconds the organ remained in a state of extreme contraction (*tetanus uteri*), but oscillations soon began again and a gradual relaxation followed. Cushny shows also that the hypogastric contains inhibitory fibres, and in one exceptional case (a pregnant rabbit) stimulation of this nerve induced pure inhibition, the uterus ceasing to contract at all. Moreover, in the virgin cat the effect of hypogastric stimulation was inhibitory, the organ undergoing relaxation. On the other hand, in the cat during pregnancy, or as a general rule after pregnancy, hypogastric stimulation led to strong and immediate contraction just as in the rabbit. It is supposed, therefore, that the inhibitory fibres prevail in the virgin, but that during and after pregnancy the action of the motor fibres conceals their presence. Cushny (1906) and Dale (1906) have dealt with the action of various drugs on the uterus. The effects of temperature upon uterine contraction were first described by Runge (1878), who found that hot water increased contraction followed by paralysis, while cold water produced tetanus. Helme (1891) obtained results which were mostly similar. Kurdinowski (1904) found that cold excited contraction to tetanus, and that long-continued mechanical stimulation produced exhaustion. Asphyxia did not cause contraction, and experimental anaemia had no effect.

Fellner (1906) states that the "nervi erigentes" are motor for the longitudinal muscles of the uterus and for the circular muscles of the cervix, but are inhibitory for the circular muscles of the uterus and the longitudinal muscles of the cervix. According to the same author the hypogastric nerves are motor for the circular muscles of the corpus uteri and for the longitudinal muscles of the cervix, but are

inhibitory for the longitudinal muscles of the uterus and for the circular muscles of the cervix. Labhardt (1906) describes an extensive system of nerves in the uterus of man and of the rabbit, the main trunks lying between the middle layer of muscles and giving off intrafascicular bundles. Keiffer (1908) describes sympathetic ganglia in the uterine and vaginal walls in the course of the large nerves coming from the hypogastric plexus.

Dembo (1885) obtained evidence for a peripheral nerve centre for the uterus in the upper part of the anterior wall of the vagina in the rabbit. Stimulation of this centre produced a very distinct contraction of both uterine cornua.

According to Jacob (1884) there is an inhibitory centre for uterine contraction situated in the medulla oblongata. This assertion is based on experiments upon rabbits, in which it was found that stimulation of the medulla caused the movements of the uterus to cease. Moreover, it is to some extent borne out by the fact that the "pains" of labour can often be inhibited by emotions and other contemporary actions of the central nervous system.

It is well known that uterine contraction can be induced by the presence of a foreign body in the uterus, by injection into the rectum, by the application of

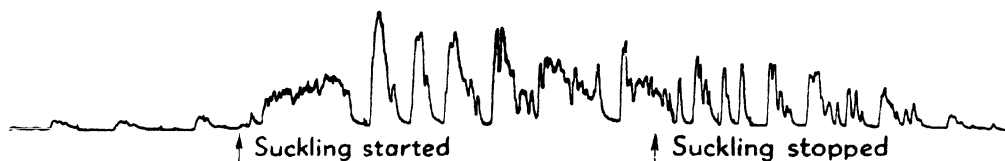


Fig. 19. 1—Tracing made at end of first week of puerperium by the intra-uterine bag method, showing effect of suckling. Time is marked in minutes. (From J. Chassar Moir, 1935.)

the child to the breast (Fig. 19. 1), and by various other means. According to Kurdinowski (1907) the sensation of any violent pain may cause uterine contraction in animals, and the organ may respond to remote stimulation (e.g. in the ears). These observations alone are sufficient to show that the contraction is often a reflex act. The experimental evidence cited above shows no less clearly that the controlling centre is in the lumbar portion of the spinal cord. Nevertheless there are many indications, as just mentioned, that the movements of the uterus can be brought under the influence of a higher centre situated in the brain. On the other hand, the fact that rhythmical contractions can continue to occur in the absence of all nervous connections is a certain proof that they are primarily independent of the central nervous system, although normally they are to a large extent influenced by it. It must be concluded, therefore, that the power to contract and relax rhythmically is an inherent property of the uterus.

The question as to the nature of the mechanism involved in uterine contraction is inseparably connected with the further problem concerning the part played by nervous influence in controlling the course of parturition.

From the above account it would appear that little is as yet definitely known about the physiology of uterine contraction as it occurs normally in the body. But, in recent years a more complete study of the anatomy of the uterine nerve supply has been made, and this has been described by Reynolds (1939). It is concerning the precise functions of the different sets of nerves and the respective

parts they play in correlation with the endocrine organs that physiologists are still to a large extent ignorant. For, as Reynolds himself says, with few exceptions, every reproductive and sex function about which we know can be subserved without the agency of these nervous structures.

The basis for the last statement is seen in Reynolds' description of uterine innervation. He states that the focal point of the uterine innervation is the pelvic plexus. Practically all the nerves passing to or from the part in question of the reproductive tract go by way of this felt-work of nerves. Its large size presumably indicates the importance of the plexus which also innervates the bladder, rectum, urethra and vagina. There is a sacral component of the plexus which contains sympathetic components. The main sympathetic pathways, however, reach the pelvic plexus from the coeliac plexus, the pre-aortic or intermesenteric plexus, and the superior, middle, and inferior hypogastric plexuses. The superior hypogastric plexus represents the caudal continuation of the pre-aortic plexus below the inferior mesenteric artery. The inferior hypogastric plexus consists of several communicating nerves on each side of the sacrum.

There are two features of the uterine cervical innervation found so far in all mammals, namely, an extramural plexus of nerves rich in ganglia, and a juxtamural plexus lying subperitoneally next to the muscular tissue of the vagina and cervix (with masses of ganglia). The existence of ganglia in the corpus uteri still seems to be doubtful, but there are definitely some along the attachment of the uterus to the broad ligament. The nerve pathways within the uterus are still very imperfectly known.

Keiffer (1932) has demonstrated three types of nerve distribution to and within the uterine muscle cell. First, there are axis cylinders giving off fine fibres which surround the muscle cells. Secondly, there are nerve terminations in the sarcoplasm of the muscle cells, with button-like expansions. And thirdly, there are axones arising from other axones and passing from sensory corpuscles of different types of structure (cones, tufts, spindles, etc., all described by Keiffer). The rich innervation of the blood vessels of the uterus has also been described by Keiffer who has extended the work of Langley and Anderson referred to above.

Reynolds says that there are no published data showing the course of the sensory nerves from the uterus to the central nervous system, and the causes of painful stimuli of uterine origin are not known. Uterine contractions *per se* do not give rise to painful sensation, but in cases where uterine contractions are painful the pain may be relieved by reducing the strength of the contractions, as by administering progesterone to women suffering from "after pains." Moir (1939) reported certain experimental investigations bearing on the nature of the pain of labour, and suggested that the immediate cause of the pain was the stretching of the muscle fibres of the lower uterine segment and cervix, rather than the contraction of the muscle of the upper segment.

IV. THE NORMAL COURSE OF PARTURITION IN THE HUMAN FEMALE

The increased size of the foetus, together with the accumulation of the amniotic fluid, causes the uterus towards the end of pregnancy to become considerably distended. The enlargement is still further increased by the growth of the uterine wall itself. Meanwhile, the altered hormone concentrations in the blood cause

the uterine muscle to become more and more responsive to stimuli. In consequence of these changes the waves of contraction which were present at the beginning of pregnancy, or even previously, as above described, become much more marked, but are still unaccompanied by painful sensation. With the onset of labour, however, these unconscious painless contractions are replaced by others of increasing intensity, and in the human subject distinctly affecting consciousness and giving rise to severe suffering. These are the "labour pains" which bring about the dilatation of the cervix uteri and lead to the expulsion of the child followed by the placenta.

At the commencement of labour the contractions do not occur oftener than once every half or quarter of an hour, but they soon become more frequent, and recur eventually at intervals of two or three minutes. Their average duration is about a minute, though actual pain is experienced for a shorter time (Sellheim, 1906a and b; Galabin, 1910; Williams, 1930). Polaillon (1880), and Schatz (1886) have shown from tracings that the period of increase occupies the main portion of the "pain," its acme being of short duration. In animals possessing bi-cornuate uteri the contractions are said to be peristaltic in nature, but this is not so evident in the case of the human subject. The contractions of the dog's uterus at term were described in some detail by Rudolph and Ivy (1930), and a similar study was made of the monkey's uterus by Ivy *et al.* (1931). The latter workers state that in the uterus simplex of the monkey concentric waves of contraction start from a quiescent area slightly ventral and cranial to the insertion of the tubes, pass medially to meet in the midline and cranial border of the uterus, then pass caudally to involve the lower segment and cervix uteri.

The clinical course of labour and the muscular forces concerned in the process are fully dealt with in the text-books on midwifery (Sellheim, 1906a and b; Galabin, 1910; Williams, 1930), and it is not proposed in the present work to devote more than a very brief space to the consideration of this subject. It is customary to divide the period of labour into three stages.

The first stage is characterised by the dilatation of the cervix and os uteri. Galabin gives the following account of the mechanical processes which take place in the uterus during this stage of labour:

"There are three elements in the mechanism of dilatation of the cervix and os; first, the mechanical stretching by the bag of membranes; secondly, the contraction of the longitudinal fibres of the uterus, which draw the cervix open; and thirdly, the physiological relaxation of the circular fibres, which is always associated with the contraction of the body of the uterus. It follows from the principles of mechanics that the effect of any given pressure within the bag of membranes in producing a tension of the edge, either of the internal or external os, is directly proportional to the diameter of the os, and therefore vanishes when the os is very small. Hence, if the os is closed to begin with, some dilatation by the stretching influence of the longitudinal fibres must have taken place before the mechanism of dilatation by the bag of membranes or parts of the foetus can come into play. The mechanical action of the dilating part, as it is pressed into the cervix, is that of a wedge; a fluid and uniform wedge, in the case of the bag of membranes; a solid and irregular wedge, in the case of the head or other part of the foetus. It follows that the effect produced by the wedge varies according to the acuteness of its angle at the points where it is in contact with the edge of the os. . . . It follows that the dilating force vanishes when there is no projection, and becomes greater the more

complete is the projection. It follows also that it becomes progressively more and more effective in proportion to the degree of dilatation which has already been produced."

The second stage, which has been called the expulsive stage, may be said to include the period from the complete dilatation of the os uteri to the delivery of the foetus. When the os has become fully expanded, and the membranes have ruptured, there is generally a short lull in the pains of labour. At the end of the lull the contractions of the uterus begin to recur with increasing vigour and frequency, while the abdominal muscles which are brought into play for the first time exert on the uterus an additional extrinsic force similar to that exerted on the rectum during defaecation. These abdominal contractions are synchronous with those of the uterus, and therefore, like them, tend to occur rhythmically. At the commencement of the process the patient is able to some extent to control the contractions by an effort of the will, but later on they are quite involuntary. The combined effect of the contractions is to drive the child, usually head foremost, through the vagina and then out through the vulva, these, however, playing a purely passive part in the act of expulsion. Very rarely, the membranes do not rupture before birth, and the child is born surrounded by a "caul."

The third stage of labour comprises the expulsion of the placenta. After the delivery of the child the uterus ceases to contract for a longer or shorter period, at the end of which its activity is renewed once more. At this time the placenta becomes completely separated from the wall of the uterus, and passes into the upper part of the vagina. It is expelled thence through the action of the muscles of the abdomen. During this stage there is almost invariably a certain amount of haemorrhage, which is represented in normal cases by from three to four hundred cubic centimetres of blood.

The duration of labour shows considerable variation, but is generally longer in primiparous women (i.e. those who have never borne children before) than in multiparous ones. The average for the former is rather more than eighteen hours, the three stages respectively occupying sixteen, two, and from a quarter to half an hour. The average for multiparous women is twelve hours, eleven of which are occupied by the first, and one by the second stage. The duration of labour in primiparous women depends also upon age, being usually more prolonged in elderly subjects.

Williams (1930) has discussed the question as to the amount of force exerted at each "pain" in a woman during delivery. He states that the expenditure of energy necessary to restrain the head of the child as it emerges from the vulva is represented by not more than fifty pounds, although the obstetrician sometimes finds it impossible to hold it back at the acme of the pain. Schatz (1895) made an attempt to arrive at a more exact estimation by inserting into the uterus a rubber bag connected with a mercury manometer. He found that whereas the intra-uterine pressure between the contractions was represented by a column of mercury of 20 mm., during the pains it rose to a height of from 80 to 250 mm.

More recent observations of a similar kind by Bourne and Burn (1927) and by Moir (1936) show that intra-uterine pressure during labour pains is equivalent to about 105 mm. mercury (Fig. 19. 2). Nearly half of this is accounted for by the "bearing-down" efforts of the mother, and pressures during the first stage of labour, before the abdominal muscles are brought into play, are therefore of the

order of 60 mm. mercury. It is an interesting finding that nearly equal pressures can be recorded during the painless uterine contractions which occur towards the end of pregnancy (Moir, 1936). By assuming that the area of foetal head on which these pressures act measures 78.5 square centimetres, Moir has calculated that the total expulsive thrust on the foetus is in an average case the equivalent of $24\frac{1}{2}$ lbs. After stimulation of the uterine muscle by pituitary extract this pressure was found in one case to reach 32 lbs.

(It is possible that the much higher intra-uterine pressures recorded by Schatz were due to the use of a large intra-uterine bag which, during descent of the head,

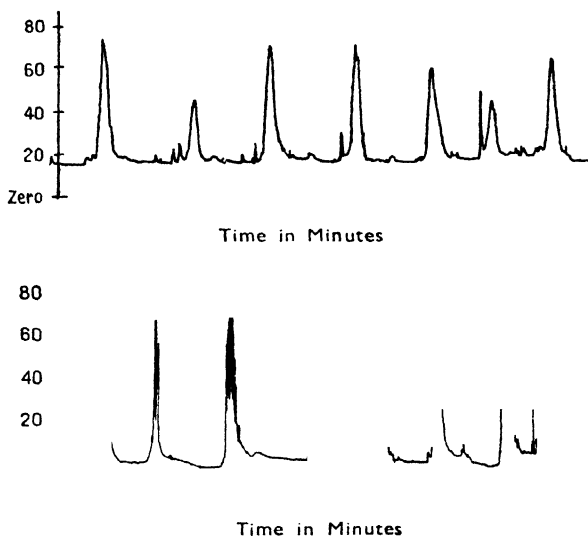


Fig. 19. 2—Tracings of intra-uterine pressure in the first and second stages of labour respectively. In B the dark up-strokes crowning the uterine contractions are due to the bearing-down efforts of the patient. Pressures are recorded in millimetres of mercury. In A the zero pressure is recorded; but in B the pressures are measured from the resting tension of the uterus. (From J. Chassar Moir, 1936.)

may have become wedged against the pelvic wall; and it is significant that Schatz's maximum readings were obtained immediately before the birth of the foetal head. The much smaller bag used by the more recent investigators was inserted high in the uterus above the presenting foetal part and was therefore more likely to give accurate recordings of intra-uterine pressure.)

V. PARTURITION IN OTHER MAMMALIA

In animals the process of delivery varies somewhat in the different kinds. In the horse, the foetus, which has been lying on its back during intra-uterine life, preparatory to birth, changes on to its side and afterwards assumes the upright position, with its muzzle and forelegs in the direction of the pelvis (Fig. 19. 3). Dilatation of the passage follows, and the foal is delivered head first (Fig. 19. 4). In

the cow and sheep the movements which occur are essentially similar. It is stated that the alteration in the position of the foetus is not brought about by its own movements, but by the uterine contractions. The revolution of the foetus prior

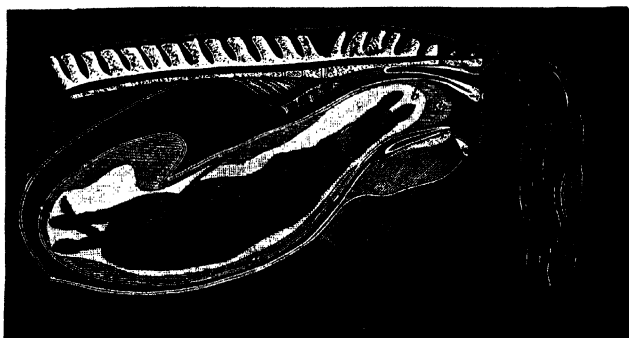


Fig. 19. 3—The first stage in the revolution of the equine foetus. The os is dilated by the membranes, which have not yet ruptured. (After Franck. From Smith, 1920.)

to birth in the mare and cow is apparently responsible for the torsion of the neck of the uterus which often occurs in these animals.

Parturition in the mare is accompanied by a complete separation of the chorion from the uterine wall. As a consequence of this fact any difficulty experienced in delivery usually causes the death of the foal. In ruminants, on the other hand,

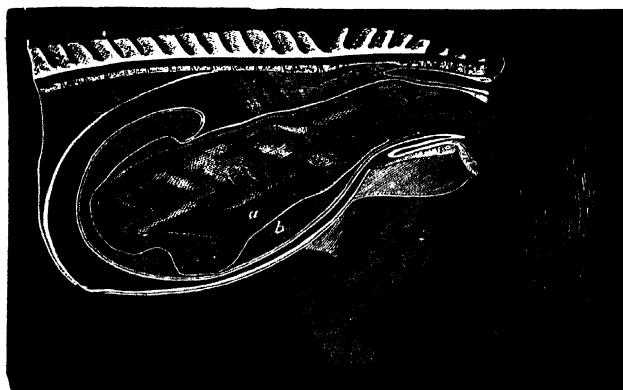


Fig. 19. 4—The foal in the normal position for delivery, the revolution being completed and the membranes ruptured. (After Franck. From Smith, 1920.)

the separation of the cotyledons takes place very gradually, so that the connection between the maternal and foetal circulation is maintained to some extent until the last. In these animals the process of parturition may last for hours (in the cow, about two hours ; in the sheep, about fifteen minutes for each lamb born). In the mare, on the contrary, delivery is usually effected very rapidly (five to fifteen minutes) (Leeney, 1896 and 1910 ; Wortley Axe, 1898 ; Smith, 1920 ; Craig, 1944).

In the sow, bitch and cat it takes from ten to thirty minutes for each young one born, with sometimes an interval of an hour between the births. The foetal membranes may be expelled with the young or be retained until a little later, when the uterus recovers its power and then expels them (in the mare several hours after the foal is born).

In the Carnivora the mother usually gnaws through the umbilical cord, but in the other animals it is torn.

In animals such as the rat, in which multiple conception is the rule, the "presentation" of the young at birth may be either "breech" or "head." The foetuses tend to be expelled irregularly, some being discharged along with the placenta, while others are born separately (Brumpt, 1907).

Hall (1947) has shown that during the last six days of pregnancy in the mouse the pubic bones move apart and a ligament 4 to 6 mm. long occupies the gap. This involves histological changes consisting of proliferation of the articular hyaline cartilage and other modifications. The gap begins to close immediately after parturition. It is probable that similar changes occur in other mammals.

Hartmann (1920) has described the phenomena attending parturition in the opossum (*Didelphys virginiana*). The very immature young make their way unaided to the mother's pouch, and in so doing crawl three inches from the vagina over an entanglement of hair; amid a forest of hair the "ten-day-old foetus" finds the nipple. Hartmann mentions that he found thirteen teats with eleven young attached.

In the actual process of being born the foetuses of the opossum do not pass out through the lateral canals of the vagina, but break through a cleft-like rupture, the "pseudo-vaginal canal," into the median canal. This occurs also in *Dasyurus* and *Perameles*. Hill (1899 and 1900) describes the lateral vaginal canals as enlarging in the pro-oestrus, containing a lymph-like fluid during oestrus when they are enormous and act as seminal reservoirs, since several days elapse between copulation and ovulation, and subsequently becoming reduced.

The young kangaroo, like the opossum, reaches the pouch unaided. (See Matthews, 1943.)

VI. THE NERVOUS MECHANISM OF PARTURITION

Parturition may be considered as being normally a reflex act, the centre of which is situated in the lumbar region of the spinal cord. On the other hand, it has been shown from experiments upon animals that the transmission of impulses through the cord is not absolutely essential to the occurrence of parturition.

Simpson (1871) removed the spinal cord from the first dorsal vertebra downwards from a number of sows a few days before parturition was due. Some of the animals died as a result of the operation, but in others parturition proceeded normally, excepting that in each case the last foetus of the series was not born.

"The uterine contractions proceeding from fundus to cervix were sufficient to expel the foetuses from the uterus; and each foetus as it came into the vagina was thence extruded by the force transmitted from the foetus behind it; but when the last foetus came into the vagina it remained there, because there was nothing to transmit the uterine expulsive force, while the vaginal and abdominal muscles, being under the influence of the spinal nerves, had been rendered powerless by the removal of the spinal cord."

Riemann (1871) states that after the destruction of the cord of a cat from the third dorsal vertebra downwards the animal gave birth to a kitten two days subsequently, shortly before its death.

Rein (1880) describes experiments upon rabbits in which he severed the uterus from all nervous connection with the cerebrospinal system, and found afterwards that the mechanism of labour was not destroyed.

Furthermore, Oser and Schlesinger (1872), as a result of experimental evidence, state that parturition can occur in animals after the severance of the sympathetic nerves which pass to the uterus, but it is difficult to understand how this operation could have been made complete without interfering with the blood-supply to that organ.

More recently, Goltz and Ewald (1896) have described an experiment in which they completely excised the spinal cord of a bitch from the mid-dorsal region downwards, and found that after the operation the animal experienced normal "heat," became pregnant, and in due course produced a litter of pups. Krueger and Offergeld (1907) have also shown that parturition is possible after destruction of the cord. Goltz (1874) had already shown that parturition could occur after the transection of the spinal cord in the dorsal region, and consequently after all connection with the higher centres had been cut off.

The last-mentioned fact is also demonstrated in the various cases in which parturition has proceeded normally in women suffering from paraplegia from the level of the mid-dorsal part of the spinal cord downwards. Routh (1898) has recorded several such cases, and in all of them labour set in and proceeded regularly (or almost regularly), but without sensation. In Routh's own patient the injury was in the dorsal region of the cord, which was completely disorganised at the seat of the fracture of the spine, as the post-mortem evidence showed. In the lumbo-sacral region, however, there were a large number of cases which were normal in appearance, so that it could not be contended that the centre for parturition had been destroyed. Routh also refers to Brachet's case (1837), which he states is the only one recorded in which the spinal lesion was apparently in the lumbar region of the cord. In this case the uterus failed to make the normal contractions, and the child was eventually extracted with forceps. The placenta also had to be removed by hand. It is clear, therefore, that a spinal lesion in the lumbar region may result in materially weakening the action of the uterus, and so may hinder the normal course of labour. On the other hand, in those cases in which the lesion was in the dorsal part of the cord, the possibility of spinal reflexes in the lumbar region could not be excluded.

Many instances have been recorded of labour having progressed "normally" after administration of a spinal anaesthetic (Whitehouse and Featherstone, 1923). It appears, however, that the abolition of the abdominal contractions tends to cause a delay in the second stage of labour; and there is also some evidence that the circular muscle fibres of the uterus do not relax and that the uterus, although active, exerts less expulsive force than usual. These authors state that stimulation of the sympathetic trunks causes the circular fibres of the uterus to contract and the longitudinal fibres to relax, while stimulation of the hypogastric nerve has the opposite effect. It is also stated that the cervical musculature responds in a contrary fashion to that of the body (Fig. 19. 5).

The following general conclusions regarding the nervous mechanism of parturition are based largely on those of Routh. (1) The act of parturition is partly automatic and partly reflex, these actions corresponding in the main to the first and second stages of labour respectively, the spinal reflexes usually commencing as soon as the membranes have ruptured and the foetal head begins to distend the vagina. (2) Direct communication with the brain is not essential for the proper co-ordination of uterine action, but it appears to exercise a controlling influence of some kind. Emotions often become a hindrance to the progress of parturition, and it would seem possible that this inhibition of uterine contractions may be brought about by the stimulation of a centre in the brain. It is also possible, however, that such inhibition is due to the liberation of adrenalin which substance

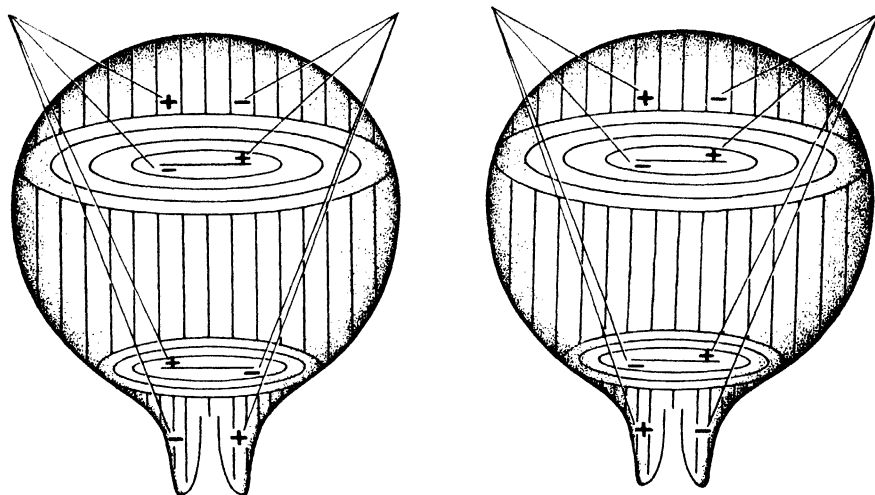


Fig. 19. 5—Innervation of the uterus according to Fellner (1906) and Whitehouse and Featherstone (1923).

is known to inhibit uterine contractions in the human female. (3) Direct communication between the uterus and the lumbar region of the cord is generally essential for the occurrence of these rhythmical contractions which take place during the progress of normal labour. There is, however, clinical and experimental evidence that the uterus is sometimes able automatically to expel its contents, at least as far as the relaxed portion of the genital cord, even when entirely deprived of all spinal influence. Routh also discusses post-mortem parturition, but points out that in most of those cases where it occurred, the expulsion of the foetus was caused by increased abdominal pressure due to putrefactive gaseous distension during a condition of muscular relaxation. There are some facts which go to prove that uterine contractility and retraction may continue or even commence after death, possibly resulting from the movements of the imprisoned child. (For further references to the literature of the nervous mechanism of parturition, *see* Bechterew (1908).)

VII. CHANGES IN THE MATERNAL ORGANISM

The influence of parturition upon the metabolism of the maternal organism is dealt with by Sellheim (1906b). There is a slight rise of temperature during the process, and the pulse rate is also affected, being much quicker during the pains, but slower between them, the difference amounting to as much as thirty-six beats a minute. The blood pressure is higher. The blood shows a marked leucocytosis, and the sedimentation rate of the erythrocytes is usually increased to a moderate degree (Bland *et al.*, 1930; Dodds and Telfer, 1930). Urine is secreted in smaller quantities, and is liable to contain traces of renal epithelium, red and white blood corpuscles, and albumen.

VIII. THE CAUSE OF BIRTH

Foster (1891) has written as follows :

“ We may be said to be in the dark as to why the uterus, after remaining for months subject only to futile contractions, is suddenly thrown into powerful and efficient action, and within it may be a few hours, or even less, gets rid of the burden which it has borne with such tolerance for so long a time. None of the various hypotheses which have been put forward can be considered as satisfactory. We can only say that labour is the culminating point of a series of events, and must come sooner or later, though its immediate advent may sometimes be decided by accident.”

What Foster wrote about this problem is now only partly true, but although progress has been made towards its solution no complete answer has yet been given to the question as to the immediate cause of parturition.

Williams (1930) has classified the various theories which have been formulated under twelve heads. These may now be briefly considered in the order adopted by him :

(1) The increasing irritability of the uterus, as manifested by its greater tendency to respond to stimulation in the later stages of gestation, is probably a factor in determining the time of birth. As already described, the uterine contractions towards the close of pregnancy are not only more frequent, but they are also much more intense. This growing irritability is no doubt to be associated in part with the increase in the size of the foetus.

(2) It is suggested that the mere distension of the uterus must, after a certain point, lead to a reaction, when the organ attempts to reduce itself to its former size, and so expels its contents. This idea receives some support from the fact that twin pregnancies and hydramnios (or the presence of an excessive quantity of liquor amnii) often result in premature labour.

(3) It has been supposed from early times onwards that parturition might be brought about through the pressure of the foetus producing a gradual dilatation of the cervix. Williams, however, has pointed out that this condition of the cervix cannot be the sole factor, since in a certain number of cases, especially in twin pregnancies, a pronounced dilatation has been known to occur for a considerable period prior to the onset of labour.

(4) Keilmann (1891), working upon the bat, came to the conclusion that the onset of labour was caused by the increasing pressure set up by the lower distended

portion of the pregnant uterus (the lower uterine segment) upon the surrounding nerve ganglia. Supposing this conclusion to be correct as applied to the bat, it is not quite clear that it is equally applicable to the human female and to other animals.

(5) Simpson (1871) and others were of the opinion that the "pains" of labour were the indirect result of a partial separation of foetus and decidua, brought about by the fatty degeneration of the latter in the last stages of pregnancy, so that the foetus became virtually converted into a foreign body, which caused the uterus to respond accordingly. It is true that part of the maternal placenta undergoes degenerative changes towards the end of pregnancy, but there is no evidence that this by itself is sufficient to cause a separation of the foetus from the uterine wall.

(6) There is no evidence in support of the theory that the exciting cause of parturition is an accumulation of carbon dioxide in the blood, beyond the fact demonstrated by Brown-Séquard (1853), Keiffer (1896), and others, that uterine contractions can be induced experimentally by this means.

(7) Spiegelberg (1891) put forward the theory that parturition was brought about through the action of substances secreted by the foetus and passed into the maternal blood. He suggested, further, that the exciting substances were elaborated as the result of an insufficiency of nutrition, and were an indication that the mature foetus required other substances than that supplied to it through the placenta. At the time when this theory was advanced there was little or no experimental basis for the statements which were made although they were not opposed to known facts. The part of the theory dealing with the supposed source is now of historical interest only; but with the passage of time more and more evidence has accumulated to show that Spiegelberg's hypothetical substances are realities which play a highly important part in the maintenance of pregnancy and the initiation of parturition. These matters will be further discussed in the next section.

(8) Tyler Smith (1849), Minot (1889), Beard (1897), and others have held the view that there is a connection between parturition and menstruation, the two processes being physiologically homologous. According to this theory, there is an increased tendency towards uterine contractions at the periods at which menstruation would occur if the condition were not one of pregnancy. Thus, Tyler Smith says that there is in all women a greater tendency to abort at the times represented by the catamenial periods. According to Minot, the menstrual and gravidal changes follow the same cycle of events, the pregnant uterus passing through a prolonged and intensified "menstrual cycle." Consequently, it is probable that there is a common cause for the ending of the series (the casting off of the superficial part of the mucosa *in both cases*). This theory has been further elaborated by Beard, who arrived at the conclusion that parturition takes place at the time it does in order that a new ovulation may be carried into effect. This author lays great stress upon the rhythmical character of the sexual processes, and points out in support of his theory that "heat" and ovulation frequently ensue shortly after parturition. That this does not happen in many animals has been already shown in an earlier chapter of this work. Moreover, Beard's theory makes no attempt to explain why parturition should occur in some animals at the close of one particular ovulation interval (e.g. in the human species at the close of the tenth), and in other animals at the termination of a different one (that is to say,

no explanation is given of the variation in the number of ovulation intervals which are comprised in the period of gestation in different animals). It cannot be said, therefore, that Beard's hypothesis as to the time occupied by gestation and the cause of birth is an adequate one.

(9) Von der Heide (1911) published some observations which he believed could be explained by assuming that an excessive quantity of foetal antigen entered the maternal blood stream towards the end of pregnancy, and that the interaction between this substance and the existing blood antigens liberated substances which initiated labour.

(10) Various writers, such as Geyl (1881), have laid some stress upon the belief that parturition occurs in women at a time which has proved, after long ages, to be the most suitable for the perpetuation of the race. A similar statement might of course be made about any other existing species of mammal, for it is only another way of stating the generally accepted belief that parturition, like all other natural phenomena in the animal world, is under the control of natural selection. In support of this contention, as applied to the human species, it has been pointed out that when labour takes place after an abnormally prolonged gestation, it frequently results in dead children, while, on the other hand, premature labour results in puny, ill-developed children, who often perish in early life.

(11) Eden (1897), and also Williams, have pointed out

“ That the frequent occurrence of infarct formation (i.e. a certain kind of degenerative change) in the placenta at term must be regarded as evidence of its senility, and that this change is analogous to the obliteration and atrophy of the chorion laeve at an earlier period. Where these changes are marked the nutrition of the foetus must be interfered with, and it is possible that certain of its metabolic products may result in stimulation of the uterine centre.”

This theory should be compared with that advanced by Spiegelberg.

(12) Lastly, Williams calls attention to the fact that excessive physical fatigue, sudden jars or violence, mental emotion, fright, etc., may lead to the termination of gestation in women. Similarly, it is well known that circumstances of a like nature may induce abortion in animals.

Williams remarks that in all probability the onset of labour in most cases is due to a combination of a number of the above-mentioned causes. The main objection to all the theories which have so far been advanced is that they take no account of the complexity of the problem. An hypothesis may be fairly adequate as a general explanation of the duration of gestation, while at the same time taking no account of the immediate cause of birth. Thus, it is no doubt true that the time of parturition is determined largely by the necessities of the offspring, but this conclusion provides no sort of explanation as to why it is that the process in any one particular species generally commences at a certain fixed stage of development and it remains for us to assume that it is one of the inherent properties of the uterus and correlated organs in the species in question that it should be so. Even on this assumption it is impossible to avoid concluding that there must be some definite exciting cause.

Some light is thrown on this question by the study of the condition of pseudo-pregnancy. The three animals in which it is best known are the dog, the marsupial cat, and the rabbit (the latter only under experimental conditions). In each of

these species during pseudo-pregnancy there is a persistent corpus luteum which at or near the end of the period enters into a state of involution and so probably is in a condition which is not dissimilar to that of the corpus luteum verum at the end of gestation. Furthermore, all these animals at the termination of pseudo-pregnancy (in the rabbit about the eighteenth day) display habits or instincts which are usually associated with the act of giving birth. Thus, the bitch may prepare a bed as if for a litter of pups, the female *Dasyurus* cleans out her pouch as if for the reception of young, and the doe rabbit plucks her breast of fur and uses it to line a nest. Since these habits are displayed at the end of pseudo-pregnancy, which, as we have seen, is dependent on the persistence of the corpus luteum, it is reasonable to suppose that the processes associated with actual parturition after true pregnancy are correlated similarly with changes in the ovarian internal secretions. These matters will now be discussed in more detail.

IX. THE HORMONAL CONTROL OF THE UTERUS DURING PREGNANCY AND PARTURITION

Since 1901, when Fraenkel and Cohn showed that the removal of the corpus luteum caused abortion in the pregnant rabbit, an immense amount of experimental investigation has been directed to the subject of hormonal control of the uterus in pregnancy and parturition. This work has been dealt with in detail in Chapter 16. Only the main facts relating especially to parturition need be considered here.

Ancel and Bouin (1912) supposed that in the first part of pregnancy the uterus tolerated its contents because of the inhibitory influence of the corpus luteum on the muscular activity of the organ, but that in the second half the inhibition was due to the presence of the myometrial gland (see p. 323). Hammond (1917) has shown that in the rabbit it is much more probable that the corpus luteum is the responsible agent throughout the whole of gestation. Corner and Allen (1929) were able to maintain gestation in oöphorectomised rabbits by injection of purified corpus luteum extract (progesterin). Knaus (1930) demonstrated that excised strips of rabbit uterus were insensitive to posterior pituitary extract if the animal had previously received injections of corpus luteum extract, or possessed functional corpora lutea in the ovaries. (See also the later memoir by Knaus (1934) where full references to the relevant literature are given.) Knaus (1929) found that with the entire rabbit after injections of luteal extracts the uterus failed to contract, as it usually does, when posterior pituitary extract was injected. Robson and Illingworth (1931) confirmed these results, finding that in spayed rabbits in which slight progestational changes were brought about by injecting luteal extracts the reactions of the uterus to oxytocic and pressor fractions of the pituitary were suppressed. They found also that a placental extract likewise had a similar inhibitory influence (cf. Robson, 1940). Allen and Reynolds (1935) demonstrated similar effects by experiments in which the contractions of the rabbit uterus were recorded *in vivo*.

It appears certain, however, that the importance of the corpus luteum in maintaining gestation varies in different animals. Thus, in the human species there are well authenticated cases in which both ovaries have been removed during pregnancy without interfering with the normal course of gestation or parturition.

Ask-Upmark (1926) has collected records of cases in which double oöphorectomy was performed at various stages of pregnancy, even during the first month, without ill effect. For this and other reasons it is supposed that in certain mammals the placenta elaborates a hormone which is capable of replacing the progesterone normally produced by the corpus luteum.

Thus the onset of labour may be due in part to the lessening of the restraining influence of the hormones of the corpus luteum and of the placenta. Further, Snyder (1934), by injecting rabbits at the 25th day of gestation with an extract of pregnancy urine (prolan), whereby a fresh set of corpora lutea was produced, was able to prolong gestation to the 40th day, eight days longer than the normal duration of pregnancy. Teel (1926) had previously noted that daily administration of bovine anterior pituitary extract to rats caused prolongation of pregnancy for from two to six days, and Evans and Simpson (1929) and Nelson, Pfiffner and Haterius (1930), by making injections or daily implantations of pituitary or luteal extracts, were also able to extend gestation in some cases for 150 hours. Furthermore, Levin, Katzman and Doisy (1931) found that chorionic gonadotrophin, free from oestrogen, maintained luteal activity and consequently gestation in rats for some days, but the young, which were apt to be unduly large, sometimes died in the uterus.

Fels (1926), Aschheim and Zondek (1927) and Smith (1927), have shown that in the human female the oestrogen content of the blood and urine is increased during pregnancy. The concentration rises steadily until just before parturition, and then rapidly falls, so that after three or four days it has reached the level for a non-pregnant woman. It appears that the oestrogen is secreted by the placenta, for removal of the ovaries does not materially alter its concentration in the urine. Cohen, Marrian and Watson (1935) have shown that urinary oestrogen consists of two fractions, one free and biologically active, the other combined as a monoglucuronide. The combined oestrogen shows a sudden fall a few days before onset of labour, while the proportion of the free form shows a correspondingly sharp rise. (Fig. 19. 6.) These workers suggest that this remarkable alteration in the relative concentrations of the two forms may be caused either by an increased production of free oestrogen, or by a sudden change in the body mechanism whereby the free form can no longer be utilised and is consequently excreted in the urine.

Smith, Smith and Pincus (1938), while noting the increase in the total urinary excretion of oestrogen until shortly before parturition, found that whereas the output of oestrone rises right up to delivery, terminating with a sharp rise at the end, the less potent oestriol rises continuously until about three days before delivery, and then rapidly falls. Many attempts have been made in the human female to induce labour by intermuscular injection of oestrone or of oestradiol benzoate, but thus far the results have been negative, or at least inconclusive (Bourne, 1935).

Reynolds (1937) believes that oestrogen acts on the uterus by co-ordinating the contractions of individual muscle fibres or bundles, thus making the organ as a whole capable of contracting rhythmically, and of responding to stimuli. Robson (1933) has shown that excised uterine muscle both of rabbits and of humans is inactive, and non-responsive to posterior pituitary extract in early pregnancy, but becomes increasingly active and responsive as pregnancy advances. He showed, also, that oestrone amplifies the reaction of the uterus to oxytocin *in vitro*, besides increasing its spontaneous activity *in vivo*. It has been suggested (Dixon

and Marshall, 1924) that labour may be precipitated by the liberation of posterior pituitary secretion or oxytocin into the blood stream. In support of this suggestion Dixon and Marshall (1924) record experiments showing that injected extract of ovaries obtained from non-pregnant animals (and in the absence of corpora lutea) appeared to promote the secretion of an active posterior pituitary substance into the cerebro-spinal canal, since after such injections the cerebro-spinal fluid had a stimulating effect upon strips of excised uterine muscle suspended in Ringer's solution. On the other hand, extract from ovaries of pregnant animals (with corpora lutea) had no such influence. The experiments of Knaus (1930 and 1934)

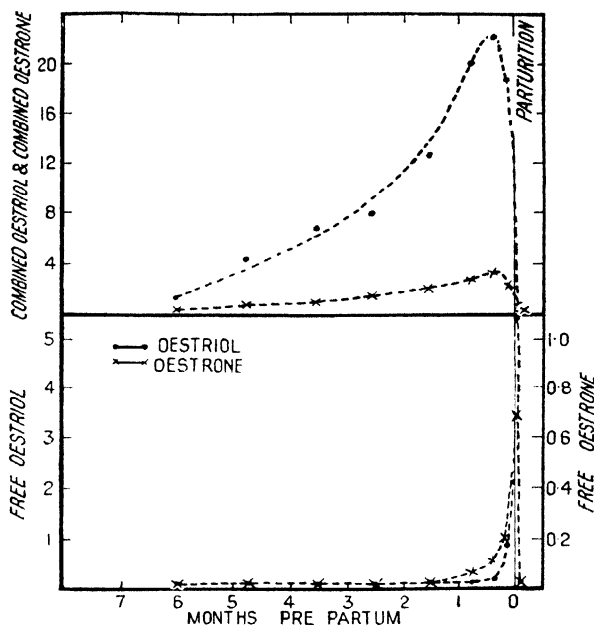


Fig. 10. 6—Excretion of oestrogen (average curves). The figures relate to milligrammes excreted per 24 hours. (From Cohen, Marrian and Watson, 1935.)

referred to above, in which an antagonistic action between posterior pituitary and luteal hormone is demonstrated afford a partial confirmation of Dixon and Marshall's view. Knaus found also that the action of pituitrin upon uterine contraction steadily increased as pregnancy advanced. In the rabbit, which has a gestation period of 30 days, pituitrin did not disturb the connexion between the uterine wall and placenta before the eighteenth day, and it was not until the last three or four days that delivery of the foetus could be induced. These results explain the observations of Sauerbruck and Heyde (1910) who united pairs of pregnant rats parabiotically so that the two rats of a pair had a common circulation, and found that one partner might give birth without the other being affected, the presumption being that the uterine muscles of the two animals of a pair were at different functional levels. In three cases, however, normal birth in one partner coincided with the abortion of partially developed foetuses in the

other. However, the physiological importance of the oxytocic hormone is indicated by the results of the experiments by Haterius and Ferguson (1938), and Ferguson (1941), who have shown that stimulation of the pituitary stalk of anaesthetised post-partum rabbits produced a marked increase in uterine activity through the action of the oxytocic factor from the posterior pituitary. (Cf. Robson, 1940; Burroughs, 1945.) Ferguson (1941) described reflex secretions of posterior pituitary hormone as being elicited by mechanical stimulation of the reproductive tract. He considered that such a mechanism may be concerned in normal parturition. Moir (1944) found that on the intact human uterus the effect of the oxytocic factor steadily increased as pregnancy advanced to term.

Harris (1947) has added to these results. He has applied the new method of remote control stimulation. A small secondary coil is buried in an animal and from this coil electrodes are led to the tissues to be stimulated. After recovery from the preliminary operation stimulation may be performed on the conscious animal by placing the part carrying the coil in an electromagnetic field. In the rabbits used there was no ill-effect from the implanted unit up to three years. He showed that the uterus of the oestrous or oestrogenised rabbit responded to stimulation of the neurohypophysis with a marked tetanic contraction, followed by an increase in frequency and amplitude of the rhythmic contractions, although the uterus of the anoestrous or pseudo-pregnant rabbit, or of the rabbit treated with progesterone, was shown to be insensitive. The converse procedure has been used by Fisher, Magoun and Ranson (1938), and Dey, Fisher and Ranson (1941), who found interruption of the nerve supply of the posterior lobe of the pituitary gland in cats and guinea-pigs might produce dystocia or difficult birth. (Harris, 1948.)

On the other hand, Smith (1932), working with rats, found that complete removal of the posterior lobe of the pituitary gland does not prevent normal labour from taking place: post-mortem examination of the experimental animals confirmed the completeness of the removal of the posterior lobe, although the pituitary stalk and pars tuberalis were left intact. It is possible, however, that oxytocin may still be present after the removal of the gland. Nevertheless, it must be stated at present that while administration of extract of posterior pituitary increases the strength and frequency of uterine contractions, there is no convincing evidence that the course of labour is directly dependent upon the activity of this gland. It is certain, however, that progesterone inhibits the stimulating action of oestrogen in the uterine responsiveness to pituitrin and oxytocin.

It is certain, also, that the excretion of free oestrogen is markedly increased shortly before or at parturition, and that the oestrogen augments the contractibility of the uterus in certain species, while progesterone inhibits this action (Bell and Robson, 1936, 1938). The response to oxytocin on the part of the uterus increases during gestation, reaching a maximum at parturition.

To summarise, uterine activity is largely dependent on the hormonal balance between the oestrogenic hormones, together with the posterior pituitary hormone or oxytocin, on the one hand, and progesterone and progesterone-like substances on the other; the former group of hormones increases the ability of the uterine muscle to contract rhythmically in response to stimuli, while the latter depresses this type of activity. Under normal circumstances labour is probably precipitated by alteration of the hormonal balance, although the mechanism is not yet completely

understood. In exceptional circumstances, local trauma or irritation, foetal death emotion or fright, may all play a part, and the initiation of parturition is therefore a complex process depending on the interplay of many diverse factors.

X. PROLONGED GESTATION

The duration of gestation in any one species usually varies within quite narrow limits, but under exceptional circumstances it may continue for an abnormally long period. Thus, Williams (1930) records a case of a woman with whom pregnancy extended for over eleven lunar months after the cessation of menstruation, instead of the usual ten lunar months (i.e. about 280 days). In this case typical labour pains were experienced at the end of the tenth month, but these subsided after a short time, and were not renewed until four weeks later, when they resulted in parturition. The same woman became pregnant a second time, when the period of pregnancy was again prolonged until the end of the eleventh month after the last menstruation. The children on each occasion were abnormally large and heavy at birth. Allen (1907) has recorded a number of further cases of prolonged gestation in women, the longest time chronicled being 334 days. In the English Law Courts, validity of paternity was contested in the case of *Gaskill v. Gaskill*, the defendant having given birth to a child 331 days after cohabitation. Verdict was given in favour of the defendant, the Lord Chancellor declaring that expert evidence rendered it manifest that there was no impossibility that the petitioner could be father of the child.

It is stated that inertia of the uterus is frequently associated with prolonged gestation ; but the occurrence of the latter is no doubt often brought about by other causes which are at present unknown.

Cases of prolonged gestation have been observed also among animals. Professor Ewart informed us of a mare in his possession in which the period of gestation was extended to twelve months instead of the usual eleven. Other causes of prolonged gestation in mares, and also in cows, have been recorded by Tessier (1817) and Franck-Albrecht-Göring (1901), and appear to be not uncommon. No satisfactory reason has been suggested to account for such cases, although Snyder's experiments in prolonging gestation in rabbits throw some light on the problem (*see* p. 512).

According to Pinard (1905), prolonged gestation may occur in rodents (*Dipodillus simoni*, *Meriones shawi*, *M. longifrons*, *Mus musculus*, etc.) as a result of suckling a large litter produced just previously to a second gestation, as if the development of the young during the latter were arrested by a relative insufficiency of nourishment. In some cases the period of gestation was half as long again as the normal duration.

Kirkham (1916 ; 1920) states that with white mice where more than two young are being suckled and the mother becomes pregnant the implantation of the embryos is retarded for about nine days, and until suckling ceases, and that a corresponding prolongation of the period of gestation occurs as compared with that of non-suckling females. In other cases of suckling mice, according to Kirkham, ovulation is inhibited or postponed. Kirkham and also Adler (1912), state that the mammary glands exert an inhibitory influence upon the stimuli coming from the blastula or from the corpus luteum. That the gestation period

in suckling mice is lengthened had been previously noted by Daniel (1910), while King (1913) records similar phenomena in white rats. These phenomena are interpreted as being of a protective nature to the parent organism, since mice whose litters are removed at birth usually produce young so frequently that they die of exhaustion before reaching their climacteric.

The causes of abortion or premature parturition are discussed below in the chapter on fertility.

XI. THE PUERPERIUM

In multiparous women the uterus continues to contract and relax at more or less regular intervals after the expulsion of the placenta which marks the termination of the third stage of labour. The contractions which occur at this period give rise to the sensations commonly known as the "after-pains." These may last several days but are not generally very severe after the first day. They are particularly liable to occur when the child is put to the breast, a fact which seems to indicate a nervous connection between the uterus and the mammary glands (see Fig. 19. 1).* In primiparous women the tonicity of the puerperal uterus is usually greater than in multiparous ones, so that the uterus is capable of remaining during this period in a state of almost uninterrupted retraction unless blood clots or other foreign bodies are present in the cavity, in which case the organ undergoes movements in attempting to expel them.

This tonic retraction of the uterus is an important factor in closing the blood sinuses, and so preventing bleeding. If, owing to any circumstance, the normal contraction and retraction of the uterus are interfered with, post-partum haemorrhage is liable to occur. This is not infrequently the case with white women who have migrated to the tropics, or with ill-nourished women in the slums, in whom the organ tends to become inert (Longridge, 1906). It follows from what has been said that multiparous women are more liable to post-partum haemorrhage than primiparous ones. Longridge pointed out, however, that the amount of post-partum discharge in multiparae is not as a rule in proportion to the severity of the "after-pains" and consequently that the latter cannot be ascribed simply to defective retraction on the part of the uterus.

According to Longridge the anaemic condition of the normal puerperal uterus is due partly to the effacement of the ovarian and uterine arteries which occurs when the uterus contracts.

"The reality of this occurrence is supported by an observation which can be made in many cases of Caesarian section; in this operation it is noticed that as long as the uterus remains outside the abdomen it tends to bleed, but that as soon as it is dropped back bleeding ceases. It is not the warmth of the abdominal cavity that checks the bleeding, since it may continue when the uterus is wrapped in warm towels outside the abdomen. But the mere fact of pulling up the uterus opens out the concertina, as it were, and allows blood to flow through the arteries. If the bladder is full at the end of labour, the uterus is pushed upwards, and slight loss may continue until the water is drawn off. As soon as the uterus is allowed to nestle down into its normal position the bleeding stops."

The puerperal vaginal discharge is known technically as the lochia. It varies considerably in amount in different individuals, and changes in character as the puerperium proceeds, ceasing altogether about the middle of the third week.

* This is probably mediated through the posterior lobe of the pituitary gland (see Newton, M. and Newton, N. R., *Pediatrics* (1950), 5, 726).

For the first few days it consists almost entirely of blood, which makes its way from the raw surface of the uterus and from lacerations caused during the delivery. This is the lochia rubra. After three or four days it becomes paler, owing to the dilution of the sanguineous discharge by mucous secretion. This is called the lochia serosa. During the next three days the normal colour of the lochia is brown. This change (from pale pink to brown) is said to be due to the fact that the normal acidity of the vaginal secretion has by this time become re-established. Longridge suggests that the brown colour is probably the result of the formation of some such compound as acid haematin. After about the tenth day the lochia assumes a whitish or yellowish-white colour, owing to the admixture of leucocytes and the cessation of the blood flow. It is then known as the lochia alba. In many cases, however, traces of blood may be observed for weeks, but the lochia alba consists mainly of secretions from the vagina and cervix, together with leucocytes, a few epithelial cells, fragments of decidual tissue, and crystals of cholesterol. Micro-organisms are also present in the discharge. Kronig (1907) believed that lochia obtained directly from the uterine cavity did not contain bacteria, excepting in cases in which the uterus was the seat of an infectious process ; but more recent investigations, notably by Petrova (1932), have shown that it is usual for micro-organisms to invade the uterine cavity during the first week of the puerperium : in some cases this occurs in the first twenty-four hours. The great majority of these organisms are non-pathogenic.

The average quantity of the discharge has been computed by Gassner (1862) at 1,485 cubic centimetres, or about 50 ounces. Giles (1893) estimated it as 10½ ounces (or considerably less than Gassner's estimation), and found further that in women who were accustomed to bleed freely at the menstrual periods the amount of the lochial discharge was beyond the average. According to Gassner, the discharge is generally less in women who suckle.

The uterus after delivery becomes rapidly reduced in size. This process is known as the involution of the uterus ; it is completely effected in from five to eight weeks, the greatest reduction taking place in the first few days. Thus the freshly-delivered uterus weighs on an average 1,000 grammes (or about 2 pounds), a week later it weighs only half that amount, at the close of the second week 375 grammes, and at the end of the puerperal period as little as 60 grammes (or about two ounces). Its decrease in size is such that by the 3rd week after parturition the organ is once more confined to the cavity of the pelvis proper, and cannot be felt above the symphysis.

The process of uterine involution is the result chiefly of changes occurring in the muscle walls. The account given of the changes in the uterus during the puerperium is based largely on that given by Williams (1930). (*See also Sellheim (1906a).*) The size of the individual cells becomes very markedly diminished, but there is little or no reduction in their number. Fatty degeneration does not take place in the muscular tissue. It is stated that the retraction of the muscle fibres produces a compression of the vessels, and that the comparatively anaemic condition of the puerperal uterus, especially in the earlier stages, is due to this cause. Subsequently the uterus becomes more vascular again.

The remains of the decidua which are not expelled at parturition undergo degeneration and are discharged in the lochia, leaving only the fundi of the glands and a certain amount of connective tissue from which the uterine stroma is

renewed. The epithelium is reformed from that of the glands, as shown by Friedländer (1870), Kundrat and Engelmann (1873), Leopold (1878), Kronig (1901), and others.

The placental area at the end of parturition is marked by the presence of thrombosed vessels. It is raised above the general surface of the uterine wall, and is irregular in shape. It very soon diminishes in size, its diameter being not more than two centimetres long at the end of the puerperal period, although its former position may be detected as an area slightly stained by blood pigment for several months after delivery. The regeneration of the uterine mucosa and especially the repair of the placental site has been studied in great detail by Williams (1931) whose posthumous article on this subject summarises existing knowledge. He has shown that the placental area is not absorbed, but is cast off by a process of undermining, brought about partly by a regeneration of glands in the deeper layers of the decidua, but more particularly by an ingrowth of the surrounding mucosa. The thrombosed blood vessels and decidual remains are thus "exfoliated," and the placental site is repaired in some six or seven weeks. The cervical mucosa is not shed after parturition.

Williams states that in the last month of pregnancy some of the sinuses at the placental area undergo thrombosis, but that this process becomes much more marked after the completion of labour, although many of the sinuses are simply compressed by the contracting uterine muscles without ever becoming thrombosed. According to Longridge, thrombosis is of little or no importance in assisting the haemostasis of normal labour. The thrombi are eventually converted into ordinary connective tissue by a cellular proliferation from the lining membrane of the vessels. While this change is in progress the lining membrane presents a folded appearance somewhat resembling a typical developing corpus luteum. This is especially well seen about the fourth week after parturition, but even up to the end of a year the convoluted appearance is still sometimes discernible (Williams, 1878; Helme, 1890). The lumina of the arteries become reduced in size, but the thickening of their walls, which takes place during pregnancy, is an alteration of a more permanent character. This histological change affords a means of discriminating between a virgin and a parous uterus.

Kuramitsu and Leo Loeb (1921) have studied the involution of the uterus in the guinea-pig and found that it is favoured by suckling. They regard the effect on the uterine tissues as acting directly, and not indirectly by way of the ovaries. Castration is stated to favour uterine involution in the guinea-pig.

It is now known that in the human species there is during the first 48 hours of the puerperium a great reduction in the oestrogen output in the urine (*see* p. 512). It is probable that a similar reduction of oestrogenic hormone in the blood stream is one of the causes of the rapid involution of the uterus. Moir (1944) has shown that there is also a rapid decline in the reactivity of the human uterus to posterior pituitary extract at this period.

The human cervix remains for some time after delivery as a soft flaccid structure with lacerated edges, but it gradually undergoes involution, the lumen becoming narrower (Fig. 19. 7 *a* and *b*). The vagina takes about the same time to recover as the uterus. After a first delivery its outlet remains permanently wider than before. The rugae reappear about the third week. The place of the hymen is taken by numerous small tags of tissue which become transformed into the

carunculæ myrtiformes. The condition of the labia majora and labia minora is generally flabby and atrophic as contrasted with that existing in virgin women.

The characteristic changes which occur in the breasts in connection with the secretion of milk are described in the next chapter.

The quantity of urine passed during the first two days of the puerperium is generally above the average. The urine frequently contains sugar, which may be either glucose or lactose. In the latter case it is generally believed that the sugar has been absorbed into the circulation from the changed mammary glands. When glycosuria occurs, it is probably comparable to post-operative glycosuria (*see* p. 477). Albumen may also be present in the urine during labour and in the first days of the puerperium. It is stated further that there is an increase in the amount of acetone (Scholten, 1900).

As mentioned above, a marked leucocytosis occurs during labour. According to Hofbauer (1897), this becomes still more pronounced during the first twelve hours of the puerperium, after which the number of leucocytes in the blood falls again and in a short time becomes normal. Henderson (1902) states that on the fifth day the average number of leucocytes per cubic millimetre is 12,000, whereas



Fig. 10. 7—(a) Virginal external os (human). (b) Parous external os (human).
(From Williams, 1930.)

immediately after parturition it is 21,000, as compared with about 8,000 in a normal woman. It is stated that there is a diminution in the number of red corpuscles during the first days of the puerperium, a circumstance which is commonly ascribed to the loss of blood at delivery and the lochial discharge. It is also said that the amount of haemoglobin is reduced, and that there is a relative increase in the quantity of fibrin and serum. Experiments show, however, that there is no appreciable shortening in the coagulation time of the blood, such as has been supposed to account for the thrombosis of the sinuses (Longridge, 1906). The sedimentation rate of the erythrocytes is usually increased to a moderate degree (Bland *et al*, 1930; Dodds and Telfer, 1930).

The pulse rate during the early days of the puerperium is usually stated to be somewhat below the normal, but according to Longridge such cases are unusual. Williams (1930) says that the pulse is slowest on the second or third day, after which it becomes quicker, resuming its normal rate after about ten days.

The temperature is ordinarily normal during the puerperium, the old idea that the commencement of milk secretion was associated with a rise of temperature having apparently no basis in fact, excepting in cases of infection.

Little attention has been paid to the changes which occur during the puerperal period in other animals. Strahl (1906) has shown that the Mammalia with so-called full placenta (commonly called Deciduata) can be arranged under three

groups according to the process of puerperal involution of the uterus. In the first group, to which man and monkeys belong, the epithelium is absent from the mucosa, and requires, therefore, to be reformed in the manner described above. In the second group the placenta is spread out over the inside of the uterus as in the first group, but in addition to this the inside of the organ is covered by a layer of epithelium. This arrangement is found in the Carnivora. In the Rodentia we often meet with the third form ; here, towards the end of gestation, not only is the womb covered with cell-tissue, but this epithelium also runs from the fimbriae right underneath the placenta, undermining it till it is finally only adhering to the walls of the uterus by a slender cord carrying the vessels. It is evident, therefore, that the uteri of the second and third groups will resume relatively quickly their non-puerperal appearance. The principal changes that occur are the reduction of the surface epithelium both by the casting off of superfluous parts and by the changing of larger cells into smaller ones, the advance of new epithelium to cover places that were bare (Duval, 1890) and the reduction and consolidation of the connective tissue. The latter process is effected by the cells becoming more compact, as in the bitch, or by a reduction in the amount of intercellular substance, as in the hedgehog (Strahl, 1906). The puerperal changes in *Tarsius* are said to resemble those of rodents. Except in those animals which belong to the first group mentioned the lochial discharge is either very slight or absent altogether.

The changes which take place in the uterus after abortion have been studied by Weymeersch (1911a and b), who states that the placenta may for a time persist. If the abortion is produced experimentally by the removal of the ovaries the absence of the corpus luteum results in an immediate contraction of the uterine vessels as well as of the uterine muscle wall. Fortuyn (1920) also states that the chorion survives after the death of the embryo in the mouse.

The changes which occur in connection with the formation of milk in animals are described in the following chapter.

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CHAPTER 20

LACTATION

By S. J. FOLLEY

The possession of mammary glands is an essentially mammalian character. The function of the mammary gland, which forms a necessary part of the sexual apparatus of mammals, is to secrete milk, a fluid elaborated from precursors furnished by the blood, and which constitutes the sole source of nourishment for the newly-born young, since in most species the latter can only obtain food by the act of suckling. The complex of phenomena comprising the synthesis of milk, its passage through the alveolar cell membrane, and its expulsion from the mammary gland, is called lactation. In the case of the cow and to a lesser degree the goat and the sheep, lactation has assumed great economic importance on account of the wide use of milk and milk products in human nutrition, and this has led to the development, by selective breeding for milk production, of breeds of cattle and goats the capabilities of which as regards lactation are far above those of the species in the wild state.

Mammary glands are present in both sexes though they are usually functional in the female only. That the male gland is equipotential with that of the female, at any rate in many species, is shown by the occurrence from time to time of cases of spontaneous lactation in the male (*see*, for example, Shannon, 1932) and by the fact that the hormonal induction of mammary growth and lactation in males of certain species of laboratory mammals, made possible by modern developments, is now a commonplace procedure.

The number and position of the glands varies greatly among different species, the number varying from one pair as in most species of Primates to eleven pairs as in *Centetes*. In a general sense the average number of mammary glands characteristic of a species is related to the average litter size and the needs of the young. Thus in the guinea-pig, where the normal litter size is small and the young are born in a comparatively advanced state of development so that if necessary they can fend for themselves, there are only two mammae, while in the rabbit, in which the normal litter size is larger and the newly-born young are naked and helpless and the gestation period is far shorter, there are usually eight mammae.

In species in which the mammary glands are few in number they are sometimes confined to the thoracic region (Primates—excepting some lemurs, Cheiroptera, Sirenia, elephants, sloths) while in other species (most Ungulata, Cetacea) they are restricted to the inguinal region. When the mammae are more numerous they are usually situated in two nearly parallel rows along the ventral aspect of the thorax and abdomen. In all orders of mammals except the Monotremata, each mammary gland is provided with a teat or nipple through which milk is withdrawn

from the gland during suckling. In the cow and most other Ungulata the mammae are contained within a definite milk-bag or udder, which is surrounded by a fibrous envelope and is suspended below the abdomen; each gland (four in the cow and two in the sheep and goat) is provided with a milk cistern or galactophorous sinus, communicating with the teat, in which milk accumulates between milkings.

In monotremes alone there are no teats, the orifices of the mammary glands being mere scattered pores in the skin, the exuded milk probably passing along the hairs, which in this region are arranged in bunches.

I. THE NORMAL DEVELOPMENT OF THE MAMMARY GLAND

The available information on the development and comparative anatomy of the mammary gland in various species has been comprehensively summarised by Turner (1939a).

It is convenient to consider the development of the mammary gland in the female as it proceeds during each of the following epochs in the sexual life of the animal, namely, embryonic and foetal, birth to puberty, during recurrent sexual cycles, pregnancy, lactation and finally, involution (*see also* reviews by Nelson, 1936, and Turner, 1939b).

Embryonic and Foetal Development

Many excellent studies of the development of the mammary gland during the embryonic and foetal period in various species have been made, among which may be cited those of Lustig (1915) on man, Hammond (1927) and Turner (1930, 1931) on the cow, Turner and Gomez (1936) on the goat, Rein (1882a) on the rabbit, Myers (1917a, b) on the rat, Turner and Gomez (1933a) on the mouse, O'Donoghue (1912) on the marsupial cat *Dasyurus viverrinus*, and Bresslau (1920) on various monotremes and marsupials. The following account, which mainly refers to the bovine mammary gland, is based on the afore-mentioned descriptions given by Turner.

The mammae belong to one of the three types of skin gland which have their origin as invaginations of the ectoderm. The location of the future mammary glands first clearly appears in the young embryo when first the formation of milk streaks and then mammary lines begins on either side of the ventral mid-line. The mammary lines consist of thickenings of the epidermis due to proliferation of the Malpighian layer. The appearance of centres of proliferation along the mammary lines defines the number and position of future teats, both normal and supernumerary. In many species the occurrence of supernumerary teats (polythelia) and sometimes mammae (polymastia) is not uncommon. At each such centre of proliferation a mammary bud or hillock gradually develops, very little of which projects above the surface, but which grows downwards, forcing the basement membrane into the mesenchyme tissue. Turner (1930) described sex differences in the size and shape of the mammary bud in the bovine at an early stage of development. According to him the male buds are spherical in shape and larger than in the case of the female, in which the shape is ovoid. The formation of the embryonic teat is marked by a gradual raising of the proximal

end of the mammary bud above the surrounding epithelium due to proliferation of the mesenchyme cells encompassing it. As the teat develops, the ovoid shape of the mammary bud is gradually obliterated by the pull of the epidermis and it is finally transformed into a funnel-shaped crater filled with cornified cells forming a kind of plug at the tip of the teat. The next significant stage in mammary development is the formation of the primary sprout which begins as an invagination at the proximal end of the funnel-shaped residue of the mammary bud. As development of the primary sprout proceeds, secondary sprouts form and each grows out at an angle from the end of the primary sprout. Later, tertiary sprouts develop and form the "anlagen" of the future arborescent duct system of the udder. The extent of development of the secondary and tertiary sprouts during the foetal period is, however, relatively slight.

Early in its development a lumen forms in the primary sprout, gradually proceeding from the proximal to the distal end. The distal end of this lumen is called the streak canal; nearer the gland it forms the teat cistern, while in the gland itself it forms the gland cistern. The streak canal, which forms the entrance to the teat and the lumen of which always remains narrow, is thus the last to open. In the cow each teat is provided with a single such excretory duct. On the other hand, in some species, including man, there are a number of excretory ducts in each teat which have developed from multiple primary sprouts, each such duct being now regarded (*see*, for example, Rein, 1882b; Bresslau, 1920; Hammond, 1927; and Turner, 1930), in contradistinction to the once widely accepted views of Gegenbauer (1876) and Klaatsch (1884), as a structure homologous with the single primary sprout in the cow. After complete differentiation of these structures the extent of the streak canal is defined by the continuation of the many-layered epithelium characteristic of the skin, in contrast to the two- or three-layered epithelium lining the teat and gland cisterns.

As the foetus grows, development of the teat gradually proceeds. Even shortly before birth, however, the development of the sphincter muscle which later surrounds the streak canal has not advanced beyond the stage where circularly disposed elastic connective tissue fibres are present. At this stage, differentiation of smooth muscle cells has hardly begun. The vascular zone of the teat is well developed prior to birth. The type of teat found in the bovine and in most other placental mammals may be called a "proliferation teat."

In the rat and the mouse the teats develop as a result of the epithelium surrounding the mammary bud growing downwards into the mesenchyme tissue. When the cornified "epithelial hood" (Myers, 1916) is shed there results a shallow depression or sulcus surrounding a somewhat deeply embedded teat (*see* Turner and Gomez, 1933a, for the mouse). Turner and Gomez call this an "epithelial ingrowth" teat. A third type of teat ("eversion teat") is found in many marsupials. The primary sprouts develop at the base of a pocket which, during lactation, becomes everted like the finger of a glove thus forming the teat (*see* Bresslau, 1920).

The formation of the udder begins quite early in the female bovine foetus, its structure at first consisting almost solely of mesenchyme cells which subsequently differentiate to fibrous tissue cells. Later, whorls of connective tissue cells and adipose tissue cells develop and gradually the embryonic udder

structure comes to consist largely of adipose tissue divided by septa of connective tissue. The early development of the septum which longitudinally divides the udder into two halves is worthy of note. Udder development is entirely lacking in the male foetus since the mammary primordia are situated on or just anterior to the scrotum, and the underlying mesenchyme tissue, which in the female gives rise to the pad of fatty-connective tissue which later provides room for the growth of the secretory tissue of the udder, is therefore very restricted.

At birth the female calf possesses an udder equipped with gland and teat cisterns of mature form, the development of secretory tissue being, however, confined to regions round the teats. Duct development has progressed very little beyond that apparent in the male at this stage.

Development During the Period from Birth to Puberty

There is some variation among different species as regards the extent of mammary development which occurs during this phase. In many species this period is characterised by a gradual increase in the extent of the mammary duct system, while in other species the duct growth before puberty is comparatively slight. In general, no alveolar development occurs in the prepubertal period. In species in which the mammary glands assume a definite compact form, as the bovine udder, there is a gradual growth of the latter due to an increase in the fatty and connective tissue.

The available information on the prepubertal development of the mammary gland in primates is relatively scanty but it seems clear that in the rhesus monkey the mammary duct system undergoes considerable extension between birth and puberty. Folley, Guthkelch and Zuckerman (1939) studied glands from two immature rhesus monkeys which had never menstruated. In each case the duct system extended well beyond the base of the nipple and showed a moderate degree of arborescence; no alveolar tissue was present. Turner and Allen (1933) and Allen, Gardner and Diddle (1935) also reported extensive duct growth in glands taken from prepubertal rhesus monkeys. In man, according to Turner (1939a), the mammary duct system undergoes slow and gradual development during this period but again no alveolar tissue forms. In well-nourished girls the breasts may increase in size before puberty owing to increase in the fatty-connective tissue.

Hammond (1927) obtained evidence of a progressive increase with age in the size of the udder in the prepubertal calf. There appeared to be some growth of the duct system accompanied by end bud formation, but no development of alveolar tissue. Similarly in the female goat (Turner and Gomez, 1936), the udder growth from birth to puberty is due, as in the case of the calf, to accumulation of fatty-connective tissue. Meanwhile, there proceeds a gradual increase in the length and ramification of the ducts, which are lined with two-layered epithelium. In the male, duct growth is much slower.

Turner and Gomez (1933a) have described the development of the mammary gland of the prepubertal mouse (*see also* Cole, 1933). Soon after birth, secondary sprouts become canalised and tertiary and quaternary sprouts form. Thereafter, in the female, the ducts increase in length and number due to dichotomous branching. According to Turner and Gomez the end buds which are observed at this

stage are formed of solid masses of epithelial cells and should not be mistaken for alveolar tissue.

Similar studies were made on the rat by Myers (1916, 1917b). The extent and degree of arborescence of the duct system gradually increases from the fifth to the ninth week from birth, but during the ninth and tenth weeks, just prior to the initiation of the oestrous cycles, the increase in duct development is more marked. At puberty, glandular development is so extensive that in some cases left and right members of pairs of thoracic or inguinal glands have met at the ventral mid-line.

In contrast to the examples just considered, mammary duct growth during the prepubertal period in the guinea-pig is comparatively slight. Some extension of the duct system does occur, however, in both male and female during this period, but more noticeable is the increase in gland size in the female due to the growth of the fatty-connective stroma (Turner and Gomez, 1933b).

In the rabbit (Ancel and Bouin, 1911), cat (Turner and De Moss, 1934), dog (Turner and Gomez, 1934b), hedgehog (Deanesly, 1934), grey squirrel (Deanesly and Parkes, 1933), fox (Rowlands and Parkes, 1935), and common shrew (Brambell, 1935) there is little mammary duct growth during the prepubertal period. In these species the prepubertal gland usually consists of a few primary ducts hardly extending beyond the base of the teat.

Development in the Virgin Female During the Oestrous (or Menstrual) Cycle and in Pseudo-pregnancy

There are significant differences between various species in the extent to which mammary development occurs in nulliparous females exhibiting cyclic ovarian activity. In general the extent and type of mammary development in the sexually mature virgin female is related to the type of cycle characteristic of the species in question. In many species the ovarian cycles are accompanied by cycles of mammary growth and regression upon which is often superimposed a progressive, if gradual, development of the mammary structures with successive cycles. Since some of the species (dog, fox, opossum, *Dasyurus*) to be considered exhibit cycles characterised by a prolonged luteal phase (pseudo-pregnancy), it will be convenient to deal in this section also with the mammary changes accompanying pseudo-pregnancy in those forms (rat, mouse, rabbit, ferret) in which it is not a natural feature of the cycle but can be induced by procedures such as sterile copulation or cervical stimulation. Species exhibiting regular cycles which do not normally include a pseudo-pregnant phase will first be considered. The rat and mouse will be included under this heading although their cycles are short as compared with those of, for example, the primates and the bovine, since if the duration of the cycle is considered in relation to the gestation period and the normal life-span the difference vanishes.

In contrast to the situation in most of the common laboratory animals, the primates are noteworthy in that mammary lobule-alveolar tissue appears to be developed as a result of successive menstrual cycles. Folley *et al.* (1939) in a study of mammary development in the nulliparous rhesus monkey, in which animal the mammary gland is a relatively flat structure, observed a gradual increase in the area of the mammary gland with age (*see also* Aberle, 1934). In

a series of non-pregnant monkeys, many of which were parous, the rate of increase of mammary gland area was found to be allometric as compared with general body growth (for terminology of relative growth, *see* Huxley and Teissier, 1936). The first menstrual cycles in this form are usually anovulatory and in cyclic monkeys which had never come under the influence of a corpus luteum, no alveolar tissue was found. Alveoli were, however, present in the mammary gland of monkeys which had ovulated. The development of alveolar tissue in the mammary gland of the rhesus monkey during recurrent menstrual cycles was also reported by Turner and Allen (1933). Cyclic changes in the mammary gland of the monkey during the menstrual cycle were described by Speert (1941).

In woman, according to Rosenberg (1922), the ovarian cycle is accompanied by cyclic changes in the mammary parenchyma, lobule-alveolar growth occurring during the luteal phase, followed by complete regression after menstruation. Evidence of cyclic alternations of growth and, to a greater or lesser degree, regression, was also reported by Lewis and Geschickter (1924), Polano (1924), Ernst (1925) and Luchsinger y Centeno (1927), though the validity of Rosenberg's conclusions was questioned by Dieckmann (1925) whose material indicated that during the early years of puberty the duct system is gradually laid down, after which there is gradual growth of lobules. The significance of the above-mentioned studies is rather difficult to evaluate since in most cases the material, obtained at post-mortem, was rather heterogeneous as regards age and parity of the subjects. Gershon-Cohen and Strickler (1938) in an X-ray study of the normal human breast could find no evidence of cyclic changes in the parenchyma during the menstrual cycle but they confirm Dieckmann's finding of oedema of the stroma just before menstruation.

The histology of the udder in the maiden heifer was studied by Lenfers (1907) who found that the epithelium of the finer ducts consisted of a single layer of cells. Such ducts should therefore be classified as alveolar ducts. Evidence of secretory activity in the duct epithelium was obtained by Hammond (1927) who also observed cyclic changes during the oestrous cycle. At oestrus the ducts are large and filled with secretion and the epithelial cells cuboidal; after oestrus the ducts shrink and the epithelium becomes columnar. With successive cycles the size of the udder increases as more and more fatty-connective tissue is laid down, and presumably there is progressive development of the ducts and secretory elements since Hammond demonstrated the presence of alveolar ducts in the udders of virgin heifers. It is, however, not clear from his description and figures whether or not true alveoli lined with single-layered epithelium were present.

The mammary gland of the guinea-pig at various stages of the oestrous cycle was studied by Loeb and Hesselberg (1917a, b) who reported cyclic changes in the structure of the gland associated with the ovarian cycle. Their conclusions have, however, been rightly criticised by Turner (1939a) on the ground that their material was not confined to glands from animals undergoing their first few cycles but even included glands from parous guinea-pigs. Turner and Gomez (1933b) observed growth of the mammary duct system in virgin females at oestrus, at which time a number of buds form along the ducts and the latter become filled with secretion, presumably originating from the cells of the duct epithelium.

During the luteal phase the ducts appeared shrunken. These changes were particularly marked during the initial cycles. No convincing evidence of the occurrence of alveolar development in glands taken from virgin females was obtained. Turner and Gomez conclude that in the guinea-pig the luteal phase of the cycle stimulates little or no mammary alveolar growth.

In the mouse and rat, which exhibit recurrent oestrous cycles of short duration, measured on the human time scale, most observers agree that the oestrous cycle is accompanied by cyclic changes in the mammary duct system (*see* Fig. 20. 1). Detailed studies of the changes in the mammary gland of the mouse in various phases of the life cycle have been made by Turner and Gomez (1933a), Cole (1933), Gardner and Strong (1935) and Fekete (1938). According to Cole, considerable growth of the existing duct system coincides with the first oestrus while each subsequent oestrus is accompanied by a further slight burst of growth. During pro-oestrus



Fig. 20. 1—Whole mounts of second thoracic mammary gland of virgin mouse. (a) At first oestrus. (b) At first di-oestrus. Note the elongation of the end buds and dilation of the ducts at oestrus. Alveoli are completely absent. $\times 6$.

buds form at the ends of the ducts, while in early oestrus the ducts dilate and the buds elongate. Regression was observed before the end of oestrus, and by the end of met-oestrus the duct system had returned to a condition characteristic of the previous di-oestrus. Histologically, no cyclic changes could be detected during the oestrous cycle, indicating that the oestrous proliferation was due rather to changes in existing epithelial cells at the duct endings, than to their multiplication. Observations which on the whole agree with the foregoing were made by Turner and Gomez, while Cogswell (1929), Bradbury (1932a), Gardner and Strong (1935) and Fekete (1938) also observed cyclic changes in the mammary gland during the oestrous cycle in the mouse. Bradbury's observations indicated that a succession of oestrous cycles causes a gradual cumulative growth of the mammary duct system in this species, though the observations of Gardner and Strong (1935) on five strains of mice suggest that full virginal development must be attained soon after the initiation of oestrous cycles, since they found that the duct system increases up to about the 70th day of age but that from the 70th to the 100th day little further development occurs. No alveoli were found in the glands of virgin mice provided they had never been pseudo-pregnant.

Cyclic changes in the mammary duct system of the female rat correlated with the ovarian cycle were observed by Sutter (1921) and Astwood, Geschickter and Rausch (1937). At oestrus, sprouting of the duct buds and the formation of fresh buds were observed; at the succeeding met-oestrus regression had set in. Turner and Schultze (1931) observed some lobule-alveolar formation in the mammary glands of adult virgin rats which suggests that successive cycles exert a cumulative effect on mammary growth in the rat.

In the dog, the marsupials *Dasyurus viverrinus* and the opossum, and probably the fox (Rowlands and Parkes, 1935) in which the luteal phase of the oestrous cycle is so prolonged as to be comparable with true pregnancy in duration (complete pseudo-pregnancy), development of the mammary alveoli to an extent similar to that characteristic of pregnancy (see below) occurs during the prolonged luteal phase (see O'Donoghue, 1912, for *Dasyurus*; Marshall and Halnan, 1917, and Turner and Gomez, 1934b, for the bitch; Hartman, 1923, for the opossum; and Rowlands and Parkes, 1935 for the fox). In the bitch, copious milk secretion is not uncommonly seen at the end of pseudo-pregnancy (Heape, 1906), though it is improbable that this occurs in virgin bitches at the end of the first pseudo-pregnancy. Similar mammary alveolar proliferation occurs in the ferret during pseudo-pregnancy induced by sterile copulation (Hammond and Marshall, 1930).

In species such as the mouse, rat and rabbit, in which the duration of pseudo-pregnancy induced by sterile copulation or cervical stimulation is much less than that of pregnancy (incomplete pseudo-pregnancy), the degree of mammary alveolar proliferation during the former condition is more or less comparable with that which occurs during the equivalent interval of pregnancy. In the rabbit (Ancel and Bouin, 1911; Hammond and Marshall, 1914; Hammond, 1917; Parkes, 1929; Fauvet, 1939), initiation of pseudo-pregnancy is followed by growth of the mammary ducts until adjacent glands meet, then by alveolar growth up to about the sixteenth day (see Fig. 20. 2), after which regression sets in. Similarly in the mouse (Turner and Gomez, 1933a; Cole, 1933), rat (Freyer and Evans, 1923; Schultze and Turner, 1933; Weichert, Boyd and Cohen, 1934) and golden hamster (Deanesly, 1938), mammary development during pseudo-pregnancy runs more or less parallel with that occurring during the first half of pregnancy, though Weichert *et al.* regard pseudo-pregnancy in the rat as resembling a shortened pregnancy in that they observed a secretory phase towards the end of pseudo-pregnancy.

In the rabbit and the ferret, which do not ovulate spontaneously, and thus, in the absence of copulation or other stimuli to ovulation, remain in continuous oestrus for a considerable time, the mammary glands in the mature virgin do not normally come under the influence of a corpus luteum. Ancel and Bouin (1911), who studied the changes in the rabbit mammary gland during oestrus, found that the restricted duct system previously present ramifies considerably at this time. In the mature virgin rabbit, in her first oestrus, not only was extensive duct development found, but also some alveoli lined with single-layered epithelium, which, if the animals had indeed never ovulated, would indicate that in the rabbit, some degree of alveolar development can occur in the absence of luteal influence. In the ferret, on the other hand, Hammond and Marshall (1930) report that no significant mammary development occurs during oestrus, which is somewhat surprising in view of the long duration of oestrus in the unmated ferret.

The outstanding implication of the results of the studies considered in this section is the close relationship of the corpus luteum to the growth of the mammary alveolar system. In species in which the cycle does not include a prolonged luteal phase there is little unequivocal evidence that growth of true alveoli,



Fig. 20. 2. Whole mount of mammary gland of rabbit 12 days pseudo-pregnant. N. denotes nipple. (From Parkes, 1929.)

characterised by a single-layered epithelium, occurs as a result of a succession of cycles, except in the primates.

Development During Pregnancy

Two main phases of mammary development can be distinguished during pregnancy, namely, the growth phase and the secretory phase. The essential difference between the *hyperplasia* of the mammary parenchyma, which is characteristic of the first half or two-thirds of pregnancy and the *hypertrophy* of the mammary gland, due to distension of the epithelial cells and alveolar lumina with secretion, which occurs during the later stages of this condition, is thus

emphasised. It should be understood, however, that there is no sharp division between these two phases of mammary gland development, inasmuch as there is evidence that glandular growth may proceed side by side with the gradual development of the secretory process. The above distinction has been emphasised, notably by O'Donoghue (1912), for *Dasyurus*, Turner and Gomez for the mouse (1933a), guinea-pig (1933b), dog (1934b) and goat (1936), Ancel and Bouin (1911) for the rabbit, as well as by Hammond (1927) in a study of the cow, while Roberts (1921) and Weatherford (1929) have presented histological and cytological evidence respectively that in the rat the mammary gland secretory cells reach their maximum number by about the thirteenth day of gestation. Cole (1933), on the other hand, observed growth of the mammary parenchyma of the albino mouse proceeding into lactation (*see below*). Similarly Jeffers (1935a) reported that mitotic, but not amitotic nuclear division was frequent in the rat mammary



Fig. 20. 3—Section of udder of heifer 8 months pregnant.
(From Hammond, 1927.)

gland in late pregnancy and further that the average number of cells per alveolus increased steadily during the latter half of pregnancy.

Additional evidence that secretory activity of the mammary gland begins in mid-pregnancy is afforded by the fact that a secretion containing milk constituents can be drawn at this stage from the udders of heifers pregnant for the first time (*see page 596*), and also by the work of Cutler and Lewis (1933), who were able to detect casein in the udders of nulliparous pregnant heifers after the twenty-fourth week of pregnancy but never before the twentieth week. Moreover, it has been shown that if pregnancy is experimentally terminated by abortion, milk secretion only develops if the operation is performed late in pregnancy (*see Loeb and Hesselberg, 1917b, for the guinea-pig and Drummond-Robinson and Asdell 1926, for the goat*).

A good description of mammary development during pregnancy in the heifer is given by Hammond (1927). During the first six months the main ducts sprout to form the alveolar ducts and the lobules themselves form. At the fifth month the lobules have become definitely formed but are still small, while by the sixth month they have greatly increased in size because of distension by secretion.

From this stage until parturition the gradual accumulation of secretion causes the whole gland to swell (*see* Fig. 20. 3).

The growth phase of the mammary gland during pregnancy in the albino mouse has been well described by Turner and Gomez (1933a). Following conception, formation of buds occurs along the walls of the ducts and particularly at their ends. The end buds representing the "anlagen" of the future lobes increase in length to form intralobar ducts, secondary buds later forming along the walls and at the ends of these. Gradually these elongate to interlobular ducts at the ends of which more buds form which eventually develop into alveoli. According to these authors, hyperplasia of the gland parenchyma is complete at the twelfth day after conception. Cole (1933), studying the mammary gland of the same animal claims, as indicated above, that only just before parturition does the glandular tissue become equal in amount to the stroma, while according to him the proportion of the former is greatest at twelve days *post-partum*.

Apart from the detailed studies already considered, papers in which sections or whole mounts of the mammary gland during pregnancy in various species are figured include those of Keynes (1923) and Dabelow (1934) on man, Wahl (1915) on the rabbit, Deanesly and Parkes (1933) on the grey squirrel, Deanesly (1934) on the hedgehog, Deanesly (1944) on the weasel, and Brambell (1935) on the common shrew.

Changes During Lactation

Following parturition, the mammary alveoli and lobules increase in size, due to increase in secretory activity, for a period which varies with the species (*see*, for example, Turner and Gomez, 1933a, and Cole, 1933, for the albino mouse). According to the latter, the amount of glandular tissue in the mammary gland of the mouse reaches its maximum at about the twelfth day *post-partum*, though it was not quite clear whether this was due to cellular hypertrophy or to increase in the number of cells. However, most observers (*see*, for example, Maeder, 1922, and Weatherford, 1929, for the rat) agree that mitosis is rarely seen in the mammary gland during full lactation, and since Cole found no mitoses after seven days *post-partum* it is likely that the former alternative is true. It may be noted that Jeffers (1935a) observed occasional mitoses in the rat mammary gland during lactation and estimated that the average number of epithelial cells per alveolus continued to increase throughout the suckling period. These results may indicate the replacement of the cells which have degenerated rather than continued growth of the parenchyma in the ordinary sense.

In the mammary gland of the lactating guinea-pig, Kuramitsu and Loeb (1921) observed mitotic cells in the alveoli shortly after parturition. The glands reached their maximum size two days after parturition, at which stage mitosis was no longer observed, though amitotic figures were frequent. At three weeks *post-partum*, diminution of secretory activity was apparent, and after four weeks the lobules and alveoli were visibly shrunken and lymphocytes had begun to appear in the alveoli. Turner and Gomez (1933b) made observations which, on the whole, agree with these.

The cytological changes in the mammary gland during lactation will not be considered here, as an account of the cytology of lactation is given in a later

section. The histological picture of the fully-lactating gland is illustrated in Fig. 20. 4.

Post-lactational Involution

Involutional changes in the lactating mammary gland (Figs. 20. 5 and 6) quickly follow failure to withdraw the secretion, whether due to removal of the young at weaning or to the gradual self-weaning of the young as they acquire other sources of food. In animals such as the cow and goat which are milked regularly, involution is more gradual, but does inevitably occur as is evidenced by the slow

decline in milk yield. The studies of Lenfers (1907) on the cow indicate that this decline may be due, at least in part, to gradual reduction of the amount of tissue capable of active secretion as involution slowly spreads through the gland. Likewise in the rabbit after weaning, Wahl (1915) observed that portions of the mammary gland may undergo involution while lactation continues elsewhere in the same gland.

Among studies of the post-lactational involution of the mammary gland may be mentioned those of Turner and Gomez (1933a), Cole (1933), Fekete (1938), and Williams (1942) in the mouse, Myers and Myers (1921), Kuramitsu and Loeb (1921) and Maeder (1922) in the rat, Turner and Gomez (1934b) in the dog and Turner and Reineke (1936) in the goat. Involution of the rabbit mammary gland following pseudo-pregnancy was described by Ancel



Fig. 20. 4—Section of lactating mammary gland of cat. The fat has been dissolved out leaving numerous vacuoles in the milk. A creaming effect may be seen in many of the alveoli. The protein coagulum (grey) is emphasised. 121.

and Bouin (1911). In general, cessation of suckling or milking is followed by a longer or shorter period of secretory engorgement during which resorption of the stagnant milk begins; the alveoli collapse and disintegrate and the lobules gradually disappear. Frequently, vacuolisation, pyknosis and karyolysis are observed. In the albino mouse, Cole (1933) noted the formation of epithelial masses due to fatty degeneration of epithelial cells. According to Williams (1942), the rapidly growing adipose stroma plays an important role in removing necrotic parenchymal elements. Williams studied mammary involution in mice after removal of the young at birth, at the height of secretion (10th day) and at the usual weaning time (21st day). When no suckling was allowed, secretion ceased within

24 hours and involution was complete in five days. If the young were removed when secretion was at its height, secretion continued for two days and engorgement



Fig. 20. 5—Section of mammary gland of rat showing early phase of involution in a lobule. Many of the alveoli have collapsed and in some the epithelium is forming central masses as it disintegrates. $\times 174$.

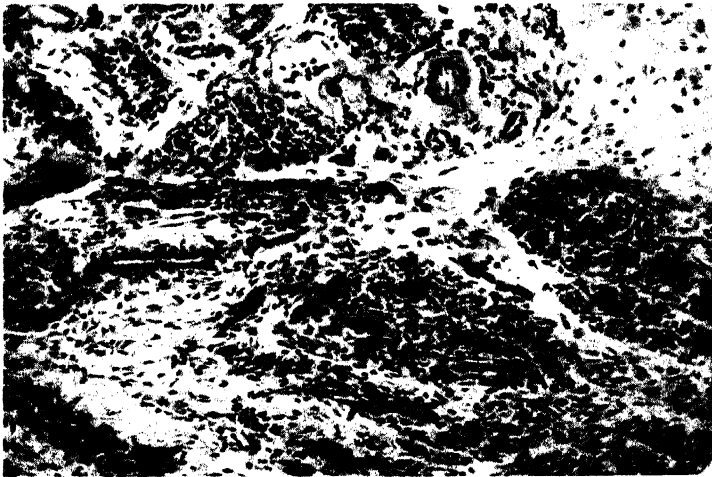


Fig. 20. 6—Section of mammary gland of rat illustrating advanced involution. Lactating alveoli are no longer recognisable and the lobular areas are reduced to disordered masses of epithelium combined with macrophages and other connective tissue cells. $\times 176$.

for as long again, the mice showing a tendency to develop mastitis. Fifteen days were required for complete involution under these conditions. When the young were weaned as usual at the 21st day, mammary congestion lasted only for about 24 hours and extensive regression occurred during the next two days.

The resting condition assumed by the mammary gland at the end of the period of involution has sometimes been described as resembling the condition of the mammary gland of the virgin (*see* Turner and Reineke, 1936, for the goat), though often the duct system is more ramified than that of the virgin gland (*see* Cole, 1933, for the mouse).

The Male Mammary Gland

In many species of mammal, the mammary gland in the male consists of a restricted duct system hardly extending beyond the base of a rudimentary teat (*see* Turner and Gomez, 1933a, for the mouse ; Turner and De Moss, 1934, for the cat ; Turner and Gomez, 1934b, for the dog ; and Turner and Gomez, 1936,



Fig. 20. 7—Whole mount of second thoracic mammary gland of male rat at 30 days illustrating the relatively extensive duct growth which occurs during the prepubertal period. $\times 10.5$.

for the goat). The observations of Turner and Allen (1933), Van Wagenen (1935), and Folley *et al.* (1939) on the whole indicate that in the rhesus monkey mammary development in the male is likewise normally very restricted though a moderate degree of individual variation in mammary gland area was indicated by the findings of Gardner and Van Wagenen (1938). In man, on the other hand, the mammary gland appears to undergo a certain amount of development at puberty (Andrews and Kampmeier, 1927 ; Jung and Shafton, 1935).

In the rat (Myers, 1917b) and guinea-pig (Turner and Gomez, 1933b) there is a certain amount of mammary duct growth in the period between birth and puberty so that the duct system in the pubertal male gland is relatively extensive (Fig. 20. 7). Further development seems to occur in the male rat at or after puberty since lobules of alveoli (Fig. 20. 8) are found in the mammary glands of mature male rats (Turner and Schultze, 1931 ; McEuen, Selye and Collip, 1936 ; Astwood *et al.*, 1937). Turner and Schultze (1931) for the rat, and Smelser (1933) for the guinea-pig reported that the male mammary gland does not regress after

castration. Contrary results for the rat were, however, reported by McEuen *et al.* (1936), and Astwood *et al.* (1937) state that acini do not develop in the mammae of castrated male rats.

In monotremes (*see* Bresslau, 1920, for *Tachyglossus (Echidna)* and *Ornithorhynchus*) the mammary glands of the male are as well developed as those of the female.

Abnormal mammary development (gynaecomastia) in the male, sometimes accompanied by milk secretion, occasionally occurs in some species. Numerous authors, for example Seifert (1920), Kriss (1930) and Menville (1933), the last two of whom give good bibliographies, have described cases of gynaecomastia in man; it appears that this condition is often associated with hyperthyroidism,



Fig. 20. 8.—Whole mount of second thoracic mammary gland of male rat at 100 days. Note the dense clusters of alveoli associated with a duct system which shows relatively little arborescence as compared with a virgin female of the same age. $\times 6.4$.

tumours of the adrenal cortex or chorionepithelioma. Cases which undoubtedly should be classified as gynaecomastia were described by Folley *et al.* (1939) in the rhesus monkey and in *Macaca nemestrina*. Such cases appear to occur with especial frequency in the goat (*see* Shannon, 1932; and Spann, 1933), though according to Turner and Gomez (1936) mammary development in the male goat is normally very restricted.

II. EXPERIMENTAL ANALYSIS OF THE ENDOCRINE FACTORS CONCERNED IN MAMMARY GROWTH

Experimental evidence has long been in existence which indicates that the development and secretory activity of the mammary gland is controlled by hormonal rather than neural mechanisms, but as will be seen in a later section, recent work suggests that nervous influences may be involved in the secretion of pituitary hormones concerned in lactation and particularly in the ejection of milk from the mammary gland.

Experiments involving severance of nervous pathways to the mammary gland, which indicated that mammary growth and lactation were largely independent of the central nervous system, were reported by Eckhard (1858), Mironow (1894), Goltz and Ewald (1896) and Pfister (1901), while Routh (1898) described the case of a woman with paraplegia below the "sixth dorsal vertebra" who lactated successfully.

Though, as is well known, the mammary glands regress after bilateral ovariectomy, this is not due to section of nervous connections, since Grigoriew (1897), Halban (1900) and Knauer (1900) were each able successfully to transplant ovarian tissue thus removed to other parts of the body, with the result that normal mammary development continued. Mammary development following the grafting of ovaries into males was reported by Steinach (1912), Athias (1915), Sand (1919), Moore (1921), Lipschütz and Krause (1923), and Gardner (1935). These experiments naturally gave rise to the belief that the ovary was concerned in mammary growth.

Lane-Clayton and Starling (1906) appear to have been the pioneers in attempts to induce mammary development by the use of organic extracts supposedly containing the relevant hormones. Their studies with aqueous extracts of foetus and placenta were followed by many others, but progress in this field was slow until the use of organic solvents for the extraction of sex hormones was introduced by Iscovesco (1912). The subsequent isolation and chemical characterisation of the sex hormones and the resulting advances in our knowledge of their physiology, described in other chapters of this book, have inevitably led to a much clearer insight into the nature of the endocrine factors involved in mammary development and lactation.

A priori consideration of the kind and degree of mammary development associated with various types of oestrous cycle, with pseudo-pregnancy and with pregnancy, would indicate that in general, the oestrogenic hormones probably stimulate duct development and that the extensive alveolar proliferation of early pregnancy and pseudo-pregnancy needs also the participation of the corpus luteum hormone, as was suggested by Ancel and Bouin (1911), Hammond and Marshall (1930), Asdell and Salisbury (1933) and others. The experimental studies now to be considered have shown that these ideas are, in broad outline, correct, though there appear to be differences between species as regards the nature of the response of the rudimentary mammary gland to oestrogens.

Oestrogen

Many species respond to oestrogen at moderate (more or less physiological) dosage levels by duct growth, often, especially when the treatment is prolonged, accompanied by a limited amount of alveolar proliferation. In other species there is evidence, much of it indirect, that oestrogen alone will evoke extensive, if not complete, alveolar development.

In the mouse, growth of the mammary ducts in response to oestrogen was observed by Turner, Frank, Gardner, Schultze and Gomez (1932), Bradbury (1932a, b), Turner and Gomez (1934a), Gardner, Diddle, Allen and Strong (1934), and Lewis and Turner (1941a). Data for the relative mammary duct growth promoting potencies of various oestrogens in mice are given by Lewis and Turner (1941b). Alveolar development, of an abnormal type, since it was localised in

certain limited areas (nodules), occurs after prolonged oestrogen administration in some strains of mice, both susceptible and resistant to spontaneous mammary cancer (Gardner *et al.* 1934 ; Gardner, 1935 ; Bonser, 1936 ; Gardner, 1941a). Prolonged treatment with large doses of an esterified oestrogen evoked extensive alveolar development resembling that of pregnancy, but again the response was abnormal in that the ducts were stunted (Gardner, Smith and Strong, 1935). Burrows (1935, 1936) has also reported alveolar development (probably localised) in the mouse following prolonged percutaneous application of certain oestrogens among which equilin seemed most effective in developing alveoli and oestrone least so. More recently, Mixner and Turner (1942b ; 1943) have observed minimal lobule-alveolar development in mice treated simultaneously with oestrone and high doses of diethylstilboestrol. Burrows' results provide some indication that various oestrogens may differ to some extent in ability to evoke mammary alveolar growth ; further investigation of the action of synthetic oestrogens from this point of view would be of interest. There seems little doubt that complete development of a normal mammary alveolar system in the mouse cannot be induced by oestrogen alone ; such alveolar growth as can be induced is of an abnormal, often precancerous, type and requires treatment with unphysiological doses for periods which amount to an appreciable fraction of the normal life-span.

In the rat, Laqueur, de Jongh and Tausk (1927) reported mammary growth in response to oestrogen but gave no morphological descriptions. Oestrogen-induced

growth of the mammary duct system was reported by Turner and Schultze (1931), Astwood *et al.* (1937) and Lewis and Turner (1941a). Turner and Schultze, however, observed slight lobule formation with the higher dosage levels used and evidence of more extensive alveolar formation was reported by Halpern and D'Amour (1934) and Nelson (1935a). Alveoli in a state of secretion were observed by Lewis and Turner (1941c) in rats treated with diethylstilboestrol but these animals were mature multiparae and the glands might possibly have contained pre-existing alveoli.

A clear description of mammary duct proliferation in the rabbit, experimentally induced by injection of follicular fluid, was given by Vintemberger (1925). More recent studies in which purified extracts or pure oestrogens were used (*see* Fig. 20. 9), on the whole indicate that in the rabbit oestrogen induces only duct growth (Parkes, 1930 ; Turner and Frank, 1930, 1932 ; Turner *et al.* 1932 ; Anselmino, Herold and Hoffmann, 1935 ; Macdonald, 1936 ; Pallot, 1936 ; Fredrikson, 1939 ; Lyons and McGinty, 1941 ; Scharf and Lyons, 1941). The findings of Frazier and Mu (1935) and Frazier and Hu (1941) who observed lactation in male rabbits following oestrogen treatment might, however, be taken as indicative of alveolar formation, though some authorities (e.g. Mixner and Turner, 1943) believe that such results may be explicable on the basis of secretion by the duct



Fig. 20. 9—Whole mount of part of mammary gland of male rabbit subcutaneously injected with 240 i.u. oestrone 5 days each week for 5 weeks. An extensive duct system has been developed but alveolar development is absent. $\times 1.5$. (From Scharf and Lyons, 1941.)

epithelium. However, alveolar formation has been reported in rabbits treated with natural oestrogen (Lyons, 1936) and diethylstilboestrol (Lewis and Turner, 1941a). There is no evidence that oestrogen alone is capable of causing *complete* mammary development in the rabbit.

In the cat (Turner and De Moss, 1934) and dog (Turner and Gomez, 1934b), oestrogen treatment seems to evoke mammary duct growth but no alveolar development. Indirect evidence (e.g. nipple growth, palpable enlargement of the mammae, secretion of milk) of mammary development in oestrogen-treated dogs was noted by Laqueur, Borchardt, Dingemanse and de Jongh (1928), Kunde D'Amour, Carlson and Gustavson (1930) and Houssay (1935b). Gardner (1941a) observed nipple growth in young female dogs treated with oestrogen but there was little if any glandular proliferation.

With the guinea-pig we pass on to consideration of species in which there is evidence that oestrogen will cause extensive alveolar development in addition to duct growth. A number of authors (Steinach, Dohrn, Schoeller, Hohlweg and Faure, 1928; Laqueur *et al.* 1928; de Jongh and Laqueur, 1930; de Jongh and Dingemanse, 1931) have shown that in the guinea-pig, oestrogen will cause nipple and mammary gland development, lactation beginning when the treatment is stopped or the dosage reduced, thus providing indirect evidence that oestrogen will cause complete mammary development in this species. Direct morphological evidence that oestrogen will cause extensive alveolar development has been presented by Turner and Gomez (1934a) and Nelson (1937) using natural oestrogens, and Lewis and Turner (1942b) with diethylstilboestrol. In the guinea-pig the mammary glands of both sexes appear to possess equal potentialities for experimental development.

In ruminants, there is much evidence, mainly indirect, that oestrogen will evoke development of alveoli in addition to the duct system. In the virgin female goat (Fig. 20. 10) treatment with oestrogen alone will cause the development of an udder capable of copious lactation (Hogreve, 1936; de Fremery, 1938; Folley, Scott Watson and Bottomley, 1940, 1941b; Lewis and Turner, 1940, 1941a, 1942a, d; Folley, Malpress and Young, 1945a; Mixner, Meites and Turner, 1944). In many of these experiments, which incidentally were mostly performed on intact goats so that the possible influence of ovarian progesterone cannot be excluded, the milk yields were so large as to justify the conclusion that extensive, though not necessarily complete, alveolar development had occurred. And indeed, histological studies of oestrogen-treated goats, including a spayed virgin, by Lewis and Turner (1941a, 1942d) and Mixner and Turner (1943) gave direct evidence of extensive lobule-alveolar development, though it could not be described as complete or normal in character. In many cases the alveolar lumen was abnormally large and the epithelium showed papillomatous outgrowths. On the other hand, it seems to be generally agreed that in the male goat oestrogen treatment causes very little udder growth (Hogreve, 1936; de Fremery, 1938; Folley *et al.* 1941b; Lewis and Turner, 1942d). Experimentally developed glands in the male goat were studied histologically by Folley *et al.* (1941b) who observed restricted alveolar formation (Fig. 20. 11). Synthetic oestrogens were used in the great majority of the above-mentioned experiments, but since copious yields were obtained by de Fremery (1938) from goats treated with oestradiol monobenzoate the results cannot be ascribed to a special property of synthetic oestrogens.



Fig. 20. 10.—Portion of complete section (100 μ) of one mammary gland of a virgin goat which had received prolonged treatment with hexoestrol. The glandular tissue, which has been stained with haematoxylin, is shown white on a black ground. Note the extensive alveolar development. $\times 1\cdot6$. The inset shows a high-power view of a portion of the alveolar epithelium. $\times 33$.

later experiments (e.g. those of Nelson and Pfaffner, 1931) with active extracts may have given misleading results (*see* Nelson, 1937) because the extracts were contaminated with unsuspected oestrogen.

However, experiments in which the effects of oestrogen alone and combined with active corpus luteum extracts (or later, crystalline progesterone) have been compared in species in which oestrogen by itself will produce little or no alveolar growth (*see* Figs. 20. 9 and 12), have shown that the combined treatment will evoke development of duct and alveolar systems, often to a degree characteristic of mid-pregnancy (*see* Turner and Gomez, 1934a, for the mouse ; Turner and Schultze, 1931, Freud and de Jongh, 1935, and Nelson, 1935, for the rat ; Turner and Frank, 1932, Asdell and Seidenstein, 1935, Macdonald, 1936, Pallot, 1936, and Fredrikson, 1939, for the rabbit ; and Turner and De Moss, 1934, for the cat). Some of these workers held that the experimental development of an extensive lobule-alveolar system requires first the application of oestrogen (presumably to develop and sensitise



Fig. 20. 12—Whole mount of part of mammary gland of male rabbit injected with 240 i.u. oestrone and 1 i.u. progesterone 5 days each week for 5 weeks. Note the extensive alveolar development. Compare with Fig. 20. 9. $\times 1.5$. (From Scharf and Lyons, 1941.)

the mammary duct system) followed by a period of combined treatment with oestrogen and progesterone. In view of later developments to be discussed below it is doubtful whether or not this hormone sequence has any real significance.

As might be expected, the synergistic action of oestrogen and progesterone is less striking in species in which oestrogen causes extensive alveolar growth. In the

guinea-pig, Nelson (1937) found that oestrogen and progesterone caused no more mammary growth than oestrogen alone, while Folley *et al.* (1939) observed no alveolar growth in immature male monkeys given short courses of treatment with oestrogen and progesterone in various ratios. In the virgin goat, however, Mixner and Turner (1943) found that combined treatment with oestrogen and progesterone caused mammary growth similar to that of mid-pregnancy, while the alveolar growth resulting from treatment with oestrogen (diethylstilboestrol) alone was variable in extent and histologically abnormal.

For optimal synergism between oestrogen and progesterone in promoting growth of the mammary alveoli, the dosage ratio in which the two types of hormone are applied appears to be of crucial importance. The work of Scharf and Lyons (1941) and Lyons and McGinty (1941) has shown that optimal prolactational mammary growth in the rabbit requires the preservation of a delicate balance (in the neighbourhood of 240 i.u. oestrone/1 i.u. progesterone) between the amounts of oestrone and progesterone administered. An excess of progesterone in relation to oestrogen inhibited alveolar growth. Studying the synergism between oestrone and progesterone in causing minimal lobule-alveolar growth in female mice, Mixner and Turner (1942a, 1943) obtained the best results with total doses of 40–133 i.u. oestrone/1 i.u. progesterone. It seems possible that

the failure of Folley *et al.* (1939) and Selye, Browne and Collip (1936a) to produce alveolar growth in male monkeys and rats respectively by simultaneous administration of oestrogen and progesterone may have been due to the use of unfavourable ratios.

As regards the mammary growth-promoting action of progesterone alone, earlier studies (*see* Turner and Schultze, 1931, Selye, Browne and Collip, 1936b, Astwood *et al.* 1937, for the rat ; Corner, 1930 ; Fredrikson, 1939, for the rabbit ; Turner and Gomez, 1934a, Nelson, 1937, for the guinea-pig) indicated that in the absence of oestrogen, progesterone was inactive. The first report to the contrary was made by Gardner and Hill (1936) who found that high doses of an oestrogen-free preparation of progesterone induced mammary duct growth in mice. More recently it has been confirmed in more than one species that provided the dosage is sufficiently great (of the order of 15 mg. daily in the rat) pure progesterone, without previous or simultaneous treatment with oestrogen, will promote growth of the mammary ducts and alveoli (*see* Selye, 1940a, b, Reece and Bivins, 1942, Selye, Borduas and Masson, 1942, Chamorro, 1944, for the rat ; Mixner and Turner, 1942a, 1943, Chamorro, 1944, for the mouse ; Hartman and Speert, 1941, for the monkey). It seems clear from these studies that the simultaneous presence of oestrogen considerably reduces the amount of progesterone required for the induction of mammary growth. And indeed studies by Mixner and Turner (1941b, 1942a, 1943) of the relative potencies of progesterone and its orally active derivative, pregnenolone, in causing minimal lobule-alveolar proliferation in mice indicate that about six times as much progesterone or pregnenolone is needed for unit response in the absence of oestrogen as when oestrogen is also given. Mixner and Turner (1942a, 1943) believe, and Petersen (1944) apparently concurs, that the explanation of this "progesterone sparing" action of oestrogen may be sought in the idea that oestrogen causes an increased vascularity and hyperaemia of the mammary stroma associated with an increased vascular permeability, all of which favour easy access to the gland tissues of mammary growth-stimulating hormones and also metabolites necessary for tissues undergoing rapid growth.

The isolation of progesterone from the adrenal cortex (Beall and Reichstein, 1938) has indicated a possible extra-ovarian source of progesterone, and it is clear that apparent species differences in the extent to which oestrogen alone will promote alveolar development in ovariectomised test animals may merely reflect differences in the extent to which the adrenal cortex is capable of producing progesterone.

Androgen

Evidence suggesting a relation between the testes and the mammary gland, at any rate in the rat (McEuen *et al.* 1936 ; Astwood *et al.* 1937), has been considered in a previous section. The probability of such a relationship has been confirmed by experiments on the administration of pure androgens which have shown that among the gynaecogenic properties known to be possessed in varying degrees by androgenic steroids may be numbered the power to develop the mammary gland. In most of the experiments the naturally occurring androgens, testosterone (either free or esterified), *cis*-androsterone and dehydroisoandrosterone have been used.

There appear to be considerable species differences in the type of mammary

growth obtained in response to testosterone. In the male mouse, testosterone caused mammary duct development (van Heuverswyn *et al.* 1939) but even in doses totalling 10 mg. evoked no alveolar development when administered to spayed females together with oestrone (Mixner and Turner, 1942b, 1943). Lewis and Turner (1939), however, observed very little duct growth in male mice with testosterone even after relatively lengthy treatment, so the possibility of strain differences in response must be considered, but their total dose was lower than that of van Heuverswyn *et al.* In the rat, on the other hand, Selye, McEuen and Collip (1936) found that testosterone caused acinar development and secretory changes in gonadectomised animals of both sexes, but it is not clear from their report whether or not duct development had occurred. Astwood *et al.* (1937) state that testosterone, even in large doses, causes no duct development in the rat but that in gonadectomised immature males and females it promotes the development of glands of restricted area with dense clusters of alveoli along the sides and at the ends of the ducts, a type of gland characteristic of the normal, mature, male rat. In mature females in which the duct system is more extensive, testosterone treatment also produces dense alveolar glands of male type but of larger area. Reece and Mixner (1939) also noted acinar development with secretory changes in the mammae of spayed adult rats treated with testosterone. In addition, mammary proliferation in the rat in response to testosterone has been reported by Nelson and Merckel (1937) and Noble (1939b) but without adequate morphological details. In the male guinea-pig, testosterone (2 mg. daily) caused some duct proliferation but no formation of alveoli (Bottomley and Folley, 1938). Interesting effects of testosterone, reminiscent in some respects of results with the rat, were reported for the male monkey by Folley *et al.* (1939). Very little, if any, macroscopically visible duct extension was caused, but microscopic study revealed a characteristic papillomatous heaping of the duct epithelium. Well-developed alveoli were present in the glands of one monkey treated for a considerable period. Somewhat similar results were reported by Van Wagenen and Folley (1939) in the ovariectomised immature female, with the difference that new alveoli were formed, in one case extensively, only in glands possessing alveoli at the outset. Again no extension of the duct system was observed, but the ducts were dilated, probably by secretion, and papillomatous outgrowths of the glandular epithelium were again observed.

Like testosterone, dehydroisoandrosterone evoked duct growth in the mouse (van Heuverswyn *et al.* 1939; Lewis and Turner, 1939) but differed in that lobule-alveolar growth occurred in the presence of oestrone (Mixner and Turner, 1942b, 1943). Negative results were obtained in the spayed female rat (receiving 1.0 mg. daily) by Nelson and Merckel (1937) and male guinea-pig (receiving 2.0 mg. daily) by Bottomley and Folley (1938). In one monkey studied by Van Wagenen and Folley (1939) dehydroisoandrosterone caused secretory changes in the duct epithelium resulting in dilation of the ducts.

By contrast, all investigators who have tested *cis*-androsterone agree that this substance, at any rate in the doses hitherto tried, produces little or no mammary growth [see van Heuverswyn *et al.* 1939, for the mouse (total dose of 8.0 mg.); Nelson and Gallagher, 1936 (1.0 mg. daily), Nelson and Merckel, 1937 (1.5 mg. daily) and Reece, 1941 (200 µg, daily) for the rat; and Van Wagenen and Folley, 1939, (1350 mg. over 60 days) for the monkey].

The action of a number of semi-synthetic androgens on the mammary gland has been studied with rather conflicting results, though in view of the results obtained with pure progesterone, discussed in the preceding section, it is obvious that negative results obtained with androgens may have been due to inadequate dosage. 17-methyl testosterone, in the presence of oestrone, promotes alveolar growth in the mouse (Mixner and Turner, 1942b, 1943) and mammary duct growth in the male guinea-pig (Bottomley and Folley, 1938). The latter investigators found another testosterone derivative, dihydrotestosterone, inactive in the male guinea-pig. Little or no duct growth was obtained in the mouse with Δ_5 -*trans*-androstenediol (van Heuverswyn *et al.* 1939) but this substance evoked extensive duct and sparse alveolar development in the male guinea-pig (Bottomley and Folley, 1938). In the rat, androstanediol caused alveolar growth and secretory changes (Nelson and Gallagher, 1936) though it was inactive in the male guinea-pig (Bottomley and Folley, 1938). Δ_4 -androstenedione caused extensive duct growth in the male mouse (van Heuverswyn *et al.* 1939), alveolar development in the rat (Nelson and Gallagher, 1936), but was inactive in the male guinea-pig (Bottomley and Folley, 1938). Androstanedione caused mammary proliferation in the rat (Nelson and Merckel, 1937).

The above results may have an important bearing on the aetiology of gynaecomastia, which is often ascribed to the production of oestrogen in the male body for which there is abundant evidence. In the light of the above findings it seems equally reasonable to suppose that this condition may in some cases be due to the abnormal production of primarily androgenic steroids.

Adrenal Cortex

The frequent association of gynaecomastia with tumours of the adrenal cortex, mentioned in an earlier section, serves as an indication that the adrenal cortex may, under some circumstances, be concerned in mammary growth, though it does not necessarily follow that specific cortical hormones are involved. The first direct evidence of the possibility of such a relationship, involving specific cortical hormones, was provided by van Heuverswyn *et al.* (1939), who showed that 11-deoxycorticosterone, an apparently specific steroid which has been isolated from cortical extracts, will promote mammary duct growth in male mice. Later, in confirmation, it has been shown that deoxycorticosterone will evoke mammary alveolar growth in the rhesus monkey (Speert, 1940a) and guinea-pig (Nelson, Gaunt and Schweizer, 1943) and, in the presence of oestrone, in the spayed female mouse (Mixner and Turner, 1942b, 1943). Also of some significance in the present connection is a case, described by Edwards, Shimkin and Shaver (1938), of gynaecomastia, following treatment with adrenal cortex extract, in a male patient suffering from Addison's disease. At the time of writing no studies of the effects of C₁₁-oxygenated cortical steroids on mammary development have been made.

Results in keeping with the foregoing were briefly reported by Nelson (1941b) who found that administration of adrenocorticotrophin evoked mammary growth in gonadectomised or hypophysectomised rats but not in adrenalectomised-ovariectomised rats, while Cowie and Folley (1944, 1947c) also observed decreased mammogenic effects of crude saline extracts of anterior pituitary in adrenalectomised-gonadectomised rats, as compared with gonadectomised controls. Nelson,

however, interpreted his results as evidence of the production of sex hormones by the adrenal.

Studies on the effect of adrenalectomy on normal mammary development have been rather conflicting. Butcher (1939) reported faster mammary development in underfed adrenalectomised rats (with or without gonads) than in controls; at autopsy the glands of the adrenalectomised animals were larger than those of the controls and showed a greater degree of arborescence. Reeder and Leonard (1944) similarly found that adrenalectomy in the rat was followed by an increase in the number of lateral duct buds but could observe no significant changes in mammary gland area. In many cases, oestrone had more effect on the glands of adrenalectomised animals than on those of intact controls. The results of Cowie and Folley (1944, 1947c), who in contrast to previous workers studied all the glands of each rat, were, however, more in harmony with the experiments on the administration of deoxycorticosterone. In quantitative studies of changes in the structure of the mammary gland they found that though adrenalectomy was not invariably followed by morphological changes, when they did occur they were always in the direction of regression.

Thyroid

There is no evidence that the thyroid hormone is capable of exerting a direct growth-promoting effect on either mammary ducts or alveolar tissue. Thus thyroid feeding caused no mammary growth in ovariectomised rats (Weichert *et al.*, 1934) and thyroxine was equally without effect in hypophysectomised rats and guinea-pigs (Gomez and Turner, 1937c).

Further, it seems clear that the mammary gland is capable of development in the absence of the thyroid, as may be inferred from the results of Bodansky and Cooke (1937) and Nelson and Tobin (1937) in the rat, Dragstedt, Sudan and Phillips (1924) in the bitch and Spielman, Petersen and Fitch (1944) in the cow, all of whom found that thyroidectomised females could become pregnant and would eventually lactate. It is improbable, however, that mammary growth proceeds to a normal extent in thyroidectomised females, for though Nelson and Hickman (1937) reported extensive experimental mammary growth in thyroidectomised rats under the influence of oestrone, Mixner and Turner (1942c, 1943) found that thyroidectomy considerably reduced the mammary lobule-alveolar growth-promoting effect of oestrone and progesterone in mice, and in the thyroidectomised cow Petersen, Knodt, Ludwick and Pomeroy (1944) found that oestrogen caused no udder growth until symptoms of myxoedema were relieved by suitable treatment. Recent morphological studies of mammary development in thyroidectomised rats, while in some respects difficult to reconcile with the results of Mixner and Turner on mice, nevertheless support the general conclusion that the absence of the thyroid profoundly modifies the morphological characteristics of the growing mammary gland. Leonard and Reece (1941), using moderately mature female rats in which the duct system at the outset was fairly extensive, found that thyroidectomy was followed by an increase in the number of lateral duct buds. Further analysis of this phenomenon (Smithcors and Leonard, 1942) indicated that thyroidectomy inhibited normal or experimentally produced duct growth but caused an increased development of alveoli. Thus, in thyroidectomised rats, oestradiol no longer showed its typical

duct growth-promoting action but resembled testosterone in causing the growth of glands with short, wide ducts but containing dense clusters of alveoli.

Additional evidence that the thyroid, as might be expected from its rôle as a general regulator of body metabolism, exerts an indirect influence on mammary development has been provided by experiments involving the administration of thyroid hormone. Weichert and Boyd (1934) reported an acceleration of the rate of mammary development and of the onset of the secretory phase in rats fed thyroid during pregnancy. Cohen (1935) found that thyroid feeding increased the degree of organisation of the mammary gland structure in the rat. In the intact mouse, Gardner (1942) observed that thyroid feeding promoted mammary duct growth, but the effect did not occur in castrated mice, which emphasises its indirect nature. Enhancement of the ability of oestrone and progesterone to promote lobule-alveolar growth in mice by administration of thyroxine was reported by Mixer and Turner (1942c, 1943) whose results suggested the existence of a broad optimum zone of thyroxine dosage for the enhancement effect. In view of this, the failure of Folley and Malpress (unpublished observations) to detect any enhancement by thyroxine treatment of the mammary growth-promoting action of hexoestrol in virgin goats may have been due to incorrect dosage.

Placenta

Comparatively little attention has been given to the possible function of the placenta as an endocrine organ concerned in mammary development or secretion during pregnancy. However, the small amount of evidence available, which exclusively refers to the rat and mouse, strongly suggests the existence of an endocrine relationship between the placenta and the mammary gland, but it is far from certain whether the growth or the secretory phase (*see* earlier section on mammary growth during pregnancy) is principally concerned.

Selye, Collip and Thomson (1935b) reported that pregnant ovariectomised rats whose uteri at autopsy contained placental tissue had well-developed mammary glands contrasting with the involuted glands of rats with empty uteri. Analogous results in ovariectomised pregnant mice which underwent foetal destruction at the twelfth day of pregnancy were obtained by Newton and Lits (1938). If the placentae were retained until autopsy on the eighteenth day, the mammae resembled those of normal mice at term, but in mice in which the placentae were aborted, the mammae had undergone unmistakable involution. Later, Newton and Beck (1939) in similar experiments showed that this action of the placenta was independent of the presence of the pituitary, but their figures hardly lend conclusive support to their contention that in many cases the presence of the placenta had caused true growth (as distinct from the hypertrophy due to secretion) beyond the condition characteristic of the twelfth day of pregnancy. That mammary regression does not follow hypophysectomy in pregnant rats and mice is shown by the occurrence of transient lactation following parturition (*see* page 557). Lyons (1944) briefly reported experiments in which oestrone and progesterone, singly or in combination, were administered to rats hypophysectomised early in pregnancy. Mammary growth only occurred in animals in which foetuses or placentae were carried to term; where resorption of the placentae had occurred the glands consisted only of ducts with a few alveolar buds. Lyons concluded that the placenta secretes a hormone which synergises with ovarian hormones in causing

mammary alveolar growth and initiates secretion. Leonard (1945) also contends that the placenta secretes a mammogenic hormone which acts synergistically with the pituitary or ovaries in causing mammary growth during the second half of pregnancy. He studied the effects on mammary development in pregnant rats of the removal at the thirteenth day of the pituitary, ovaries, foetuses and placentae, singly and in all possible combinations. The placenta was unique among the factors studied in that it was the only one of them which by itself would prevent regression of the mammary gland. In the presence of either the pituitary or the ovaries in addition to the placenta the glands at autopsy resembled those characteristic of the nineteenth day of pregnancy.

Considering all these results in the light of the generally accepted view that the phase of true mammary growth is mainly confined to the first half of pregnancy, a consideration which has largely been neglected by the aforementioned workers, it would seem that while there is some suggestion that the placenta may be involved in normal mammary growth, its chief rôle may be concerned with the initiation of the secretory phase in the latter half of pregnancy.

Anterior Pituitary

Mammary growth in hypophysectomised animals.—The studies on experimental mammary development, considered in the foregoing sections, were concerned with animals with intact pituitaries. The still controversial question whether or not ovarian hormones are effective in evoking mammary growth in hypophysectomised animals has received considerable attention in recent years. This question is important since it involves the possibility that mammary growth is due to the action of the pituitary rather than to the direct effect of ovarian hormones.

Those who first studied the effects of oestrogen alone or combined with progesterone on the mammary gland of the hypophysectomised animal, mostly agreed that sex hormones would promote mammary growth in the absence of the hypophysis (*see* Ruinen, 1932, Freud and de Jongh, 1935, and Nelson, 1935a, for the rat; Nelson, 1935b, for the guinea-pig; and Houssay, 1935b, for the dog). These results, if valid, would thus indicate that the pituitary plays no direct part in mammary development.

However, opposing reports soon followed. Already in 1935 Selye, Collip and Thomson (1935a) stated that oestrogen did not prevent mammary involution following hypophysectomy in the rat but their evidence as stated is hardly convincing. Many other workers have since reported failure to evoke mammary development in hypophysectomised animals by procedures known to succeed in the presence of the hypophysis. In the hypophysectomised mouse oestrogen was reported to be inactive by Gomez, Turner, Gardner and Hill (1937), Gomez and Turner (1937c) and Lacassagne and Chamorro (1939). Similar negative results with deoxycorticosterone were reported by Chamorro (1940) and Smithcors and Leonard (1943). In the hypophysectomised rat, negative results with oestrogen were reported by Reece, Turner and Hill (1936), Gomez and Turner (1937c), Astwood *et al.* (1937), Nathanson, Shaw and Franseen (1939), Reece and Leonard (1941), Samuels, Reinecke and Petersen (1941), Leonard and Reece (1942) and Reece and Leathem (1945); with oestrogen and progesterone by Gomez and Turner (1937c); with androgen by McEuen, Selye and Collip (1937), Noble

(1939b), Reece and Leonard (1942) and Leonard and Reece (1942); and with deoxycorticosterone by Leonard and Reece (1942). Essentially similar results with oestrogen were obtained in the hypophysectomised guinea-pig by Gomez and Turner (1936a) and Lyons and Pencharz (1936) and with oestrogen and progesterone by Gomez and Turner (1937c). The latter workers extended their studies to include rabbits, cats and ground squirrels.

In incompletely hypophysectomised animals, even when only minute pituitary remnants remain, sex hormones apparently cause marked mammary stimulation (Gomez and Turner, 1937c; Gomez, Turner, Gardner and Hill, 1937; Reece and Leonard, 1941; Leonard and Reece, 1942), so it appears that the onus of demonstrating the completeness of hypophysectomy is on any who claim that ovarian hormones will evoke mammary growth in the absence of the pituitary. The earlier claims referred to above are open to the criticism that convincing evidence of the completeness of hypophysectomy was not given, but this does not apply to the mostly more recent studies, now to be considered, in which positive results have been obtained, since these studies were based on animals in which completeness of pituitary removal has been proved by histological examination.

Growth of mammary ducts and alveoli under the influence of oestrogen and progesterone has been reported in completely hypophysectomised rabbits by Asdell and Seidenstein (1935) (*see also* Asdell, Brooks, Salisbury and Seidenstein, 1936) and Fredrikson (1939). Gardner (1940) has reported slight growth of the mammary rudiments of completely hypophysectomised male mice with oestradiol, progesterone or deoxycorticosterone, but more extensive growth with oestradiol combined with either of the other two steroids. Essentially similar results were also reported by Gardner (1941b) and Gardner and White (1942). In completely hypophysectomised rats, Leonard (1943) obtained growth of end buds with oestradiol and slight hyperplasia of duct epithelium with testosterone, while Smithcors and Leonard (1943) evoked limited mammary growth with oestrogen and progesterone, singly or in combination, only if the treatment began immediately after hypophysectomy.

There is thus some conflict of evidence as to whether or not ovarian hormones and other steroids will promote mammary growth in the absence of the hypophysis. However, in the studies in which positive results have been obtained in completely hypophysectomised animals, with the exception of that of Asdell and Seidenstein (1935), it is doubtful whether the response was as great as would be obtained in animals with intact pituitaries. The experiments of Leonard (1943) suggest that the age of the experimental animals may be a factor which affects the response.

Further evidence on this subject comes from experiments showing that in pregnant rodents, in which hypophysectomy at mid-pregnancy does not usually cause abortion, mammary growth proceeds, or at any rate the mammary glands do not regress, in the absence of the hypophysis (*see* Newton and Beck, 1939, Newton and Richardson, 1941, Gardner and Allen, 1942, for the mouse; Pencharz and Long, 1933, Jeffers, 1935b, for the rat; Desclin, 1939, for the guinea-pig; but *see also* Pencharz and Lyons, 1934, who noted reduced mammary development in three hypophysectomised guinea-pigs which carried young to term). The conclusion to be drawn from these studies is that the hypophysis is not necessary for mammary growth, or at least for the maintenance of an already

existing mammary structure (*see* page 551), so long as placental tissue is present, and it has been suggested by some who hold that the pituitary is involved in mammary development that the placenta can assume the function of the pituitary in this respect. This contention, however, requires more direct proof.

It has been suggested by Astwood *et al.* (1937) that the failure of mammary development in response to ovarian hormones, in the absence of the pituitary, may be due not to the lack of anterior pituitary hormones which directly cause mammary growth, but to a lowered state of nutrition consequent upon the operation, since they found that oestrogen caused no mammary growth in intact rats whose food intake was restricted. On the other hand, Samuels *et al.* (1941) failed to evoke mammary growth with oestrogen in force-fed hypophysectomised rats. Support to the idea that absence of mammary growth responses in hypophysectomised animals may be due, partially at any rate, to generalised metabolic effects, comes from experiments in which mammary growth has been evoked by combined treatment with oestrogen and anterior-pituitary growth hormone (Nathanson *et al.* 1939; Reece and Leonard, 1941). In general the best responses occurred in animals which gained most weight or lost least during the treatment. Against these experiments it may be objected that the growth hormone preparations were not pure and must have contained other anterior-pituitary factors, conceivably some acting directly on the mammary gland, but the responses to the growth preparation alone were either nil or very slight. Finally, we cannot escape the significance of the finding of Trentin and Turner (1941) that the threshold of response of the rudimentary mammary gland of the mouse to oestrogen was increased by inanition.

Mammogenic action of anterior-pituitary extracts.—The first direct and unequivocal demonstration of mammary growth in ovariectomised animals (rabbits) in response to anterior-pituitary extracts was provided by Corner (1930). Similar results were later reported by Asdell *et al.* (1936). Lyons and Catchpole (1933b) reported that anterior-pituitary extracts containing the anterior pituitary-lactogenic hormone, prolactin (*see* page 558), caused mammary hyperplasia in addition to secretion. However, prolactin extracts used by Gardner and Turner (1933) and Riddle, Bates and Dykshorn (1933) exhibited no mammary stimulating activity. Later, Lyons (1942) claimed to have observed local hyperplasia of the mammary epithelium in response to intra-mammary duct injections of purified prolactin in the rabbit.

A few years later, failure to evoke mammary growth with ovarian hormones in completely hypophysectomised animals led Turner and his school to postulate, as the agent directly responsible for mammary development, a hitherto unrecognised anterior-pituitary hormone (or hormone complex)—mammogenic hormone—secreted in response to stimulation by ovarian hormones. Direct evidence in support of this theory was provided by Gomez and Turner (1937c) and Gomez, Turner and Reece (1937) who produced mammary development in hypophysectomised guinea-pigs by repeated implantation of pituitaries from oestrogenised rats, pituitaries from untreated rats being ineffective. Later, extracts of anterior-lobe tissue from pregnant cows, in which the mammogenic factor would be operative, were shown to stimulate mammary growth in gonadectomised rats and rabbits while material from non-pregnant cattle was without effect (Gomez and Turner, 1938a). A method of assay of anterior-pituitary mammogenic hormone, based on

the stimulation of mammary duct growth in male mice, was developed by Lewis, Turner and Gomez (1939) (*see also* Lewis and Turner, 1939), and the mammogenic potency of pituitary tissue from various types of cattle evaluated (Lewis and Turner, 1939). In support of the mammogenic hypothesis, pituitary tissue from dairy cows was found to contain more mammogen than tissue from beef cattle, the highest content being found in tissue from lactating dairy cows. Some evidence was also provided that in accordance with the mammogen theory oestrogen treatment will increase the mammogenic potency of rabbit anterior-lobe tissue (Gomez and Turner, 1938b ; Lewis and Turner, 1939).

Lewis and Turner (1938, 1939) claimed that the mammogenic duct growth factor, unlike other known anterior-pituitary hormones, was soluble in organic solvents, which immediately suggests that the results might have been due to oestrogen present in the blood contained in the pituitary tissue. Parallel determinations of the oestrogenic and mammogenic activities of a number of anterior-pituitary extracts indicated, however, that the oestrogen content of the extracts was not sufficient to account for their mammogenic activities (Lewis and Turner, 1939). Further positive results with lipid extracts of anterior pituitary were claimed by Lewis, Gomez and Turner (1942) and Gomez (1942), but Greep and Staveland (1941) were unable to confirm the ether-soluble nature of anterior pituitary mammogen. Turner and his school (Trentin, Lewis, Bergman and Turner, 1943) have, however, more recently abandoned the claim that ether-alcohol extracts of pituitary tissue will cause mammary development ; they now believe that this property is associated with the protein fraction.

Lewis and Turner (1939) believed that some of their results suggested the presence in anterior-pituitary extracts of two mammogenic hormones, one secreted in response to oestrogenic stimulation and responsible for mammary duct growth, the other capable of evoking alveolar growth and needing for its production the additional stimulus of progesterone. Later, a more definite claim was made to have obtained evidence of the existence of a mammogenic hormone responsible for lobule-alveolar development (Mixner, Lewis and Turner, 1940). Assay of the progesterone content of these extracts indicated that the former was not present in sufficient amounts to account for the observed effects (Trentin, Mixner, Lewis and Turner, 1941). A method of assay of this factor employing ovariectomised female mice was developed (Mixner and Turner, 1941c) and since it was later shown (Mixner and Turner, 1942a) that simultaneous treatment of the test animals with oestrogen enhanced the lobule-alveolar growth-promoting potency of anterior-pituitary extracts, the method was later modified by specifying the use of oestrogenised ovariectomised mice (Mixner and Turner, 1943). This method is of course applicable to the assay of the lobule-alveolar growth-promoting activity of substances other than anterior-pituitary extracts, for example, of sex steroids. Since the original claim to have effected a partial separation of the two mammogens was based on the supposed lipid solubility of the duct growth factor, the separate existence of a second factor must remain somewhat dubious until purified preparations of both hormones have been obtained. Mixner, Bergman and Turner (1942), however, believe that the lobule-alveolar growth-promoting mammogen is distinct from prolactin, thyrotrophin and the gonadotrophins.

Mammary growth in hypophysectomised and gonadectomised animals under the influence of crude aqueous extracts of anterior pituitary has been reported by

other workers, e.g. Greep and Staveland (1941) and Cowie and Folley (1944, 1947c), but such results do not necessarily prove that specific mammogens are involved. And indeed the mammogen theory has not found universal acceptance since not only have some workers, as we have seen, succeeded in producing some mammary growth with steroids in completely hypophysectomised animals, but others have failed to confirm the claim that pituitaries from oestrogen-treated donors are particularly rich in mammogenic activity (Nelson, 1938, 1939a; Reece and Leonard, 1939). Further, it has been repeatedly shown in a number of species that mammary growth may be produced by local, percutaneous application of oestrogen and that, if the dose be low enough, growth preferentially occurs in the treated gland, the neighbouring glands either being unaffected or showing much less growth (*see* MacBryde, 1939, for women; Lyons and Sako, 1940, for the rabbit; Nelson, 1941a, for the guinea-pig; Gardner and Chamberlin, 1941, for the mouse; Speert, 1940b, and Chamberlin, Gardner and Allen, 1941, for the monkey). This phenomenon of localised mammary growth under the stimulus of doses of oestrogen incapable of exerting systemic effects (which should not be confused, as Petersen, 1944, has done, with generalised mammary growth in farm animals due to percutaneous application of supra-threshold doses) has been cited (e.g. by Lyons and Sako, 1940) as conclusive evidence against the mammogen theory, since if oestrogens act by way of the pituitary (i.e. to all intents and purposes systemically) growth would be expected in all glands alike. Mixner and Turner (1943), however, contend that the phenomenon is explicable on the supposition that such oestrogen treatment causes local hyperaemia, thereby increasing the supply, to the treated gland, of circulating mammogen already present in the blood in amounts ordinarily insufficient to cause mammary growth (*see also* Mixner and Turner, 1942a). But attempts to produce mammary growth by the local application of an irritant such as turpentine, either alone or combined with systemic injection of oestrogen, hardly produced convincing evidence in support of this explanation (Mixner and Turner, 1941a; Lewis and Turner, 1942c). That the co-operation of a pituitary factor, not necessarily mammogen, may be necessary for the localised mammary responses to oestrogen is indicated by the failure of Leonard and Reece (1942) to secure such responses in hypophysectomised animals.

From the foregoing discussion it is clear that further evidence is required before the question of the role of anterior-pituitary hormones in mammary growth can be regarded as clarified. One general conclusion seems to emerge. Notwithstanding the fact that a number of workers have successfully stimulated some degree of mammary growth in the absence of the hypophysis, the co-operation of pituitary hormones seems necessary for the experimental production of normal growth such as occurs during pregnancy. In the present state of knowledge, however, it seems best to reserve judgment regarding the existence of specific mammogenic hormones, particularly since there is some indication that certain of the already well-characterised anterior-pituitary hormones may in co-operation with ovarian hormones produce marked growth of the mammary glands in the absence of the hypophysis. Thus there is a growing body of evidence that purified preparations of prolactin, in combination with ovarian hormones, will promote mammary development in completely hypophysectomised animals perhaps to a degree unrealisable by use of the ovarian hormones by themselves (*see* Gardner and White, 1941, 1942; Gomez, 1942; and Lyons, 1943). The possibility, suggested by Mixner

and Turner (1943) that these results are due to the presence of mammogens in the prolactin preparations used must be considered in the light of the fact that prolactin can now be obtained in a state of considerable purity (*see* page 563); it is a point that, like many others arising in connection with the mammogen hypothesis, requires further study. Gardner and White (1941) suggest in explanation of their results that prolactin sensitises the mammary gland to the growth-promoting action of oestrogen, which is considered to be direct, a diametrically opposite view to that adopted by Mixner and Turner (1943) whose views may be epitomised thus: oestrogen causes the secretion, by the anterior lobe, of the mammogen responsible for duct growth, at the same time producing favourable conditions for its action by increasing the vascularity and permeability of the capillaries and perhaps sensitising the gland to mammogen stimulation; progesterone evokes the secretion of the mammogen which directly stimulates lobule-alveolar growth, oestrogen exerting a synergistic effect by continuing to influence the vascularity and growth of the stroma.

Evidence which suggests the possibility that, in addition to prolactin, anterior-pituitary adrenotrophic hormone may also play some rôle, not yet elucidated, in mammary growth has been presented by Nelson (1941b), Gardner and White (1942) and Cowie and Folley (1944, 1947).

III. ENDOCRINE FACTORS IN LACTATION

Anterior Pituitary

Effect of hypophysectomy on lactation.—Numerous experiments on the effect of hypophysectomy on lactation have provided evidence of the important rôle of the hypophysis in milk secretion. In various species it has been shown that hypophysectomy during pregnancy will prevent normal lactation even though live young are born. Failure of lactation after hypophysectomy during pregnancy was reported in the rat by Pencharz and Long (1933) and Bergman (1934), in the guinea-pig by Nelson (1935b), in the ferret by McPhail (1935a), in the cat by Allan and Wiles (1932) and McPhail (1935b) and in the dog by Houssay (1935a). Transient mammary secretion following parturition in hypophysectomised rodents has been observed (*see* Selye, Collip and Thomson, 1933c, 1934, Newton and Beck, 1939, Newton and Richardson, 1941, Gardner and Allen, 1942, for the mouse; Selye, Collip and Thomson, 1933b, 1934, for the rat; and Pencharz and Lyons, 1934, for the guinea-pig). It has been suggested in explanation of such results that the placenta may take over the function of the pituitary as regards the initiation of milk secretion (*see also* section on the rôle of the placenta in mammary growth, page 551).

Hypophysectomy during lactation invariably causes rapid and, as far as is known, complete cessation of secretion; observations in the mouse (Selye *et al.*, 1933c, 1934), rat (Selye *et al.*, 1934), guinea-pig (Nelson, 1935b), ferret (McPhail, 1935a), cat (McPhail, 1935b) and dog (Houssay, 1935a) are in complete agreement on this point.

Further, certain experimental procedures which normally cause lactation in the presence of the hypophysis do not do so in its absence. Thus neither removal of all the foetuses from the rat in late pregnancy (Collip, Selye and Thomson,

1933) nor removal from the non-pregnant rat of ovaries intensely luteinised by injections of chorionic gonadotrophin (Selye, Collip and Thomson, 1933a) results in lactation if the pituitary is simultaneously removed. Similarly, in the guinea-pig, Nelson (1935b) found that removal of functional ovarian grafts from males which had carried them for some time, or cessation of oestrogen treatment in animals of either sex, was not followed by lactation if the hypophysis was removed at the same time.

Initiation of lactation by anterior-pituitary extracts.—The experiments just considered point to the existence of a hormone (or hormone complex) of the pituitary essential for the initiation and maintenance of lactation. The first direct evidence of the existence of such a hormone was provided by Stricker and Grueter (1928) who found that injection of aqueous anterior-pituitary extracts into ovariectomised pseudo-pregnant rabbits initiated lactation. Soon afterwards the same authors reported the re-initiation of secretion in bitches which had gone dry and the stimulation of lactation in the sow and cow with anterior-pituitary extracts (Grüter and Stricker, 1929).

The ability of anterior-pituitary extracts to initiate lactation in adequately developed glands was quickly confirmed in experiments on a variety of species including the monkey (Riddle *et al.* 1933 ; Allen *et al.* 1935), virgin heifer (Catchpole, Lyons and Regan, 1933), goat (Evans, 1933), sow (Gardner and Turner, 1933), dog (Gardner and Turner, 1933 ; Lyons, Chaikoff and Reichert, 1933 ; Houssay, 1935b), rabbit (Parkes, 1929 ; Corner, 1930 ; Turner and Gardner, 1931 ; Gardner and Turner, 1933 ; Lyons and Catchpole, 1933b ; Anselmino *et al.* 1935 ; Asdell *et al.* 1936), guinea-pig (Nelson and Pfaffner, 1930 ; Lyons and Catchpole, 1933a). In many of these experiments the amounts of milk obtained were not great, certainly less than would have been secreted under conditions of normal lactation, but the response would of course depend on the extent of mammary development existing at the time of treatment, unless the extracts themselves were capable of causing mammary proliferation (*see* discussion in a preceding section).

Some of the earlier workers were of the opinion that their anterior-pituitary extracts contained a specific lactation-stimulating hormone, and the idea that a new hormone was involved was strengthened as evidence became available (Riddle *et al.* 1933 ; Gardner and Turner, 1933 ; Catchpole and Lyons, 1933) that lactogenesis was not associated with the anterior-pituitary hormones (growth, gonadotrophic and thyrotrophic hormones) comparatively well characterised at that time. Finally, Riddle, Bates and Dykshorn (1932, 1933) provided evidence that associated with the new hormone was the property of causing enlargement of and secretory activity in the pigeon crop-gland, a property of anterior-pituitary extracts first described by Riddle and Braucher (1931). This hormone now enjoys the status of a well-characterised anterior-pituitary protein hormone which is generally recognised as being concerned in the initiation and probably the maintenance of lactation (*see* Fig. 20. 13). When first discovered it was variously named *prolactin* (Riddle), *galactin* (Turner) and *mammotropin* (Lyons). Recent American practice tends to favour the terms *lactogen* or *lactogenic hormone* ; the name *prolactin* is adopted in this chapter in accordance with English usage.

Assay of prolactin.—The majority of methods used for the bioassay of prolactin involve the pigeon crop-gland response. There are many respects in which this

response is convenient for assay purposes (*see e.g.* Bates, 1937 ; Meites, Bergman and Turner, 1941a) and its discovery has undoubtedly facilitated the isolation of prolactin as a pure protein. Nevertheless, from the standpoint of the rôle of prolactin in lactation, assay methods based on lactation responses in mammals are obviously preferable for theoretical reasons, and thus the development of a suitable mammalian method is desirable if only to provide an assessment of crop-gland results. Some attempts have been made to devise mammalian assay methods, but as yet without conspicuous success. The crop-gland methods fall into three

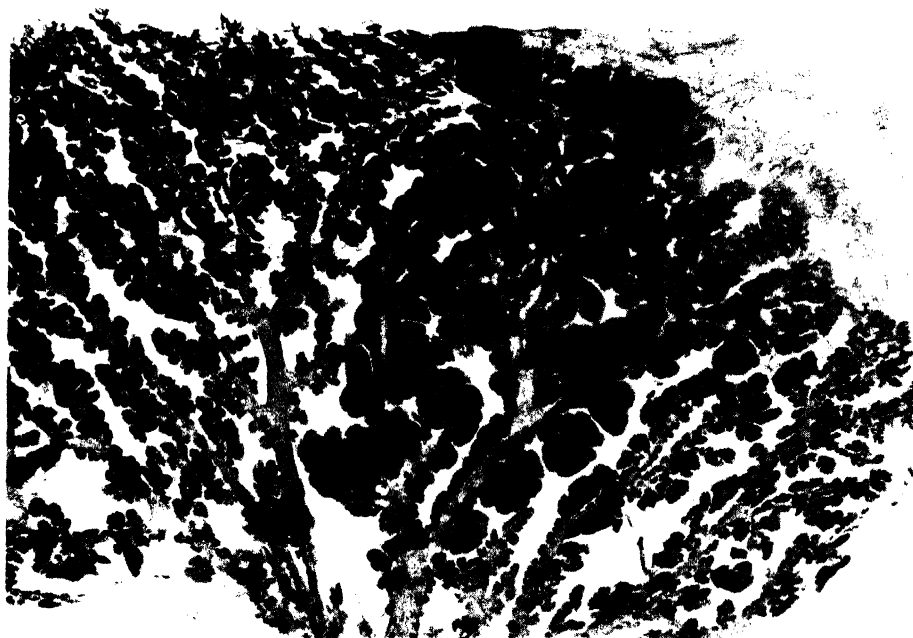


Fig. 20. 13—Portion of whole mount of mammary gland of female rabbit into one galactophore of which prolactin has been injected. Note the secretory hypertrophy in response to prolactin confined to the treated sector. 3. (From Lyons, 1942.)

main classes : “systemic” methods entailing determination of crop weights ; “systemic” methods involving minimal stimulation ; and “local” or micro methods also involving minimal stimulation. Review articles on the assay of prolactin have been published by Bates (1937), Lyons (1937b) and Riddle and Bates (1939).

Systemic methods involving intramuscular or subcutaneous injections and weighing of the stimulated crop-glands have been described by de Fremery (1936), Riddle *et al.* (1933), Dyer (1936) and Rowlands (1937), whose papers should be consulted for details. For a statistical evaluation of a typical systemic crop-weight method and a discussion of the necessary precautions, see Folley, Dyer and Coward (1940). The relationship between crop weight and the logarithm of the dose is linear or approximately so over a useful range (Riddle *et al.* 1933 ; Dyer,

1936; Evans, 1937; Wolff, 1937; Folley, Dyer and Coward, 1940). Hall (1944a) has shown that data in the lower ranges of response are fitted by a curve of the form

$$by = \log (ax + 1)$$

where y represents the dose, x the crop weight and a and b are constants, while Emmens (1940) has pointed out that the sigmoid dose-response curve (see Fig. 20. 14) may be well fitted over the whole range by a logistic equation. Many factors which affect the response have been uncovered. These include age of bird (Riddle *et al.* 1933), race (Bates, Riddle and Lahr, 1936; 1939a), environmental temperature (Folley, Dyer and Coward, 1940) and route of injection (Bates and Riddle, 1936 ;

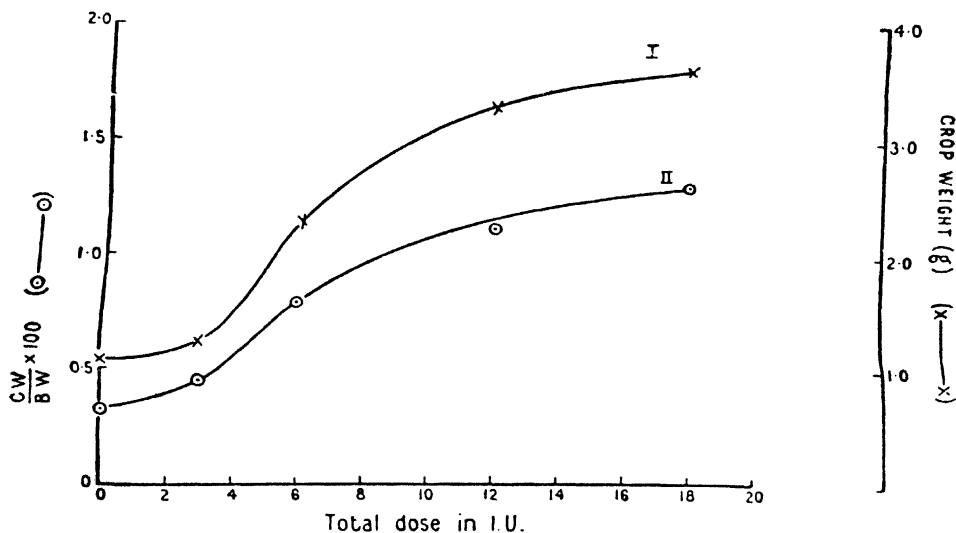


Fig. 20. 14—Response of pigeon crop-gland to prolactin. Curves showing relationship between total dose of prolactin (International Standard) and (I) mean absolute crop-weight or (II) mean crop-weight expressed as a percentage of body weight. Six daily subcutaneous injections were given followed by autopsy on the seventh day. (From Folley, Dyer and Coward, 1940.)

Lahr, Elwell and Riddle, 1941; Hall, 1944a). Birds kept in darkness during the test give the same response as birds continuously exposed to artificial light (Folley, Dyer and Coward, 1940). Seasonal variations in response to a given preparation have been reported by Bates and Riddle (1941) and confirmed by Hall (1944a). The response may be affected by the presence of inert substances (e.g. muscle extract) in the test preparations (Friedman and Hall, 1941 ; Hall, 1944a). There appears to be no appreciable sex difference in response to prolactin injected intramuscularly (Bates, Riddle and Lahr, 1939b ; Hall, 1944a) but there are indications that males give a greater response than females, particularly if the birds are immature, when the hormone is injected by the subcutaneous route (Folley and White, 1937 ; Bates *et al.* 1939b ; Hall, 1944a). The response is diminished by hypophysectomy (Gomez and Turner, 1936c ; Schooley, Riddle and Bates, 1937) and by simultaneous treatment of the birds with oestrogen (Folley and White, 1937 ; Folley, 1939 ; Bates *et al.* 1939b). Since the latter workers observed a decreased response in

fasting pigeons it is possible that these last-named effects may be partly due to reduction in food intake.

McShan and Turner (1936) described an assay method involving systemic injection of the prolactin, but in which the end-point, which was of necessity estimated subjectively, was the occurrence of threshold stimulation of the crop as determined by visual examination. The prolactin unit for this purpose was defined as the amount of hormone which, under the specified conditions, would evoke a threshold response in 50 ± 11 per cent. of a group of 20 birds. Using this method, Meites *et al.* (1941a) found that, as in the case of crop-weight methods, a smaller amount of hormone is needed per (bird) unit by the subcutaneous route than when injected intramuscularly. This type of method, since it involves subjective judgments is probably less accurate than methods involving crop weights.

An extremely sensitive method of detection of prolactin, which has been adapted to the quantitative assay of smaller amounts of the hormone than can be assayed by the methods already described, was introduced by Lyons and Page (1935). In this method small amounts of hormone are injected intradermally over the crop-sac, the end-point being the production of threshold stimulation of a small area adjacent to the injection site. In addition to its great sensitivity, this method has the additional advantage that two preparations may be compared in one bird, since the pigeon has two crop-sacs, thus eliminating differences in sensitivity among individual birds. Like the systemic minimal stimulation method the local intradermal method may be criticised on the ground that it involves a subjective judgment; however, it seems possible that, as suggested by Leblond and Allen (1937), the colchicine technique might be used to give an objective response—perhaps as soon as 9 hours after injection.

The micro method, originally used for the assay of prolactin present in urine, has been modified by Reece and Turner (1937b) for the determination of prolactin in the rat pituitary, the response being subjectively rated according to the size of the area of stimulation.

According to Bates and Riddle (1940) the response to a given dose of intradermally injected prolactin is dependent on the volume of fluid injected, so that this factor must be rigidly standardised. This observation was confirmed by Hall (1944b) but Meites *et al.* (1941a) reported that the response was independent of the injection volume over a wide range. Lahr, Bates and Riddle (1943) observed that various procedures such as plucking feathers, intradermal injection of saline as well as of solutions of various non-specific irritants, increased locally the mitosis rate of the crop epithelium and pointed out that such factors might introduce a disturbing influence into micro prolactin assays. Hall (1944b), however, considers that such artifacts are unlikely to be of much importance. Another factor which needs standardisation would appear to be the location of the injection in relation to the underlying crop-sac, since Hall (1944b) found that different parts of the crop-gland were not equally sensitive. As will be seen later, in assaying the prolactin content of pituitary tissue by the intradermal method, it is important to remember that, according to Hall (1944b), extracts give appreciably higher results than tissue suspensions.

Various estimates have been given of the magnitude of the micro (bird) unit in relation to the amounts of hormone necessary to give threshold responses by

systemic routes. The (bird) units (i.e. total amounts of prolactin required to elicit the chosen response), both micro and systemic, described by different authors obviously depend to a considerable extent on such details of technique as duration of the test, subdivision of the dose, route of the systemic injection, etc., and for such details the original papers should be consulted. Ratios of micro to "systemic" units have been given as $\frac{1}{100}$ (Lyons and Page, 1935; Bates and Riddle, 1936), $\frac{1}{100} - \frac{1}{50}$ (Chasin, 1936), $\frac{1}{1000}$ (Lyons, 1937a), $\frac{1}{10000}$ (Lyons, 1937b) and $\frac{1}{100}$ (Meites *et al.* 1941a). A table of comparisons between types of pigeon unit has been given by Bergman, Meites and Turner (1940). In the present connection it is interesting to note that Hall (1944b) reported that subcutaneous injections over the crop-gland gave results little different from those given by intradermal administration; on the other hand, Bates and Riddle (1936) state (somewhat ambiguously) that the threshold dose for a single subcutaneous injection over the crop-gland is practically the same as when four daily injections are given in a region remote from the crop.

There are two main difficulties in devising mammalian methods for the assay of prolactin. One is the difficulty of choosing a suitable end-point; the other problem is to select a type of experimental animal which is reasonably uniform in respect of the mammary tissue both as regards morphology and physiological reactivity. In most of the methods dealt with below an "all-or-none" end-point has been chosen, namely, the ability of the operator to express milk from the nipples. Rabbits and guinea-pigs have been used exclusively; rats and mice have proved relatively unresponsive to prolactin (e.g. *see* Gardner and Turner, 1933; Riddle *et al.* 1933).

Gardner and Turner (1933) described a method involving the use of pseudo-pregnant rabbits. In their method an attempt was made to obtain a quantitative measure of the response by grading the glands according to an arbitrary and subjective scale. This method has also been used with some success by Nelson (1934b). Lyons and Catchpole (1933b) suggest that the non-ovulated, non-lactating virgin rabbit, ovariectomised just prior to testing, may be superior to the pseudo-pregnant rabbit for assay purposes.

The use of the guinea-pig ovariectomised at oestrus was proposed by Lyons and Catchpole (1933a), the end-point being the ability to withdraw milk by manipulation. Nelson (1934b) criticised this method on the grounds that adult female guinea-pigs frequently lactate spontaneously after ovariectomy while immature ones are unsuitable because of insufficient mammary development. Instead, he recommended the use of pregnant guinea-pigs hysterectomised late in pregnancy. Lyons (1941) has more recently carried out prolactin tests on guinea-pigs 6-18 months old and weighing at least 650 g. If the injections were made during the first few days of the oestrous cycle approximate comparisons of the potencies of different extracts could be made. Some animals which have proved to react satisfactorily could be used repeatedly.

Comparative assays of prolactin preparations by mammalian and pigeon methods have been reported by Bergman *et al.* (1940), Meites *et al.* (1941a) and Lyons (1941). As might be expected, the minimal amounts of hormone necessary for lactation responses in guinea-pigs and rabbits are of higher order than are necessary for minimal crop-gland responses in pigeons.

It cannot be said that any assay method, based on the initiation of lactation

in mammals, of accuracy comparable to the pigeon crop-weight method or of sensitivity anywhere approaching the intradermal crop test has yet been developed. Lyons (1942) has been able to induce localised secretion in rabbit mammary glands by injections of small amounts of prolactin into single galactophores and it should be possible to develop a more satisfactory and sensitive assay method on this basis.

Purification of prolactin.—Prolactin is a protein which can be readily brought into aqueous solution but is relatively insoluble in an isoelectric region centring around pH 5.5 approx. The fact that prolactin activity is associated with an iso-insoluble substance, first recorded by Riddle *et al.* (1932), was quickly taken advantage of to prepare partially purified preparations (Riddle *et al.* 1933; Lyons and Catchpole, 1933a; Gardner and Turner, 1933) and has since proved an important factor in the preparation of the pure hormone, the first protein hormone of the anterior lobe to be isolated.

Prolactin may be extracted in acid or alkaline solution from fresh, undissected whole pituitaries or anterior lobes, or from acetone-desiccated glands. Bergman and Turner (1937), comparing four extraction procedures, found that in their hands extraction with alkaline 60–70 per cent. alcohol (Bates and Riddle, 1935) was superior to extraction with aqueous acid (Riddle *et al.* 1932), aqueous alkali (Riddle *et al.* 1933), acid acetone (Lyons and Catchpole, 1933a) or glacial acetic acid (McShan and Turner, 1935). Later (Bergman and Turner, 1942) they studied the extraction of acetone-dried gland with alkaline aqueous alcohol of various concentrations and found that 80 per cent. alcohol at pH 10–11 gave the most active product, assaying 20 i.u. per mg.

Bates and Riddle (1935) described a method of purification which has been widely used. Fresh or desiccated gland is extracted at pH 9–10 with 60–70 per cent. ethanol followed by precipitation at pH 6 in presence of an increased concentration of ethanol. The product is then re-dissolved and separated from gonadotrophin (F.S.H.) and thyrotrophin by precipitation at pH 3–4 in the presence of SO_4 . Further purification is effected by precipitation of the product from 70 per cent. ethanol at pH 6 in the presence of NaCl. The authors state that about 70 per cent. of the original hormone is obtained uncontaminated with F.S.H. or thyrotrophin. Young (1938) replaces the last stage of this method by a series of isoelectric precipitations from aqueous solutions at pH 5.5.

Lyons (1937a, b) produced a modified version of his original procedure (Lyons and Catchpole, 1933a) involving extraction of undissected sheep pituitaries with acid-acetone followed by precipitation of the prolactin by raising the acetone concentration. This method not only gives a preparation free from gonadotrophin and thyrotrophin but also is capable of removing most of the adrenocorticotrophin; the latter is precipitated from aqueous solution at pH 6.5 and the prolactin at pH 5.5. An essentially similar method was subsequently used by Li, Lyons and Evans (1940a, b, 1941a) to prepare prolactin assaying 20–30 i.u. per mg. which was found to exhibit a high degree of molecular homogeneity. Li, Simpson and Evans (1942) have recently devised another method for obtaining what is probably pure prolactin, since it is a protein exhibiting molecular homogeneity with respect to solubility, electrophoretic mobility and diffusion, from the crude powder obtained in the first stage of the Lyons acid-acetone extraction procedure. In this method prolactin is separated from its most troublesome biologically

active contaminant—adrenocorticotrophin—by alternate precipitations at pH 3 in presence of NaCl and at pH 5.6.

A novel and interesting method giving preparations assaying approximately 30 i.u. per mg. (sheep gland) has been developed by Schwenk, Fleischer and Tolksdorf (1943). This method is based on the discovery by Fleischer (1943) that prolactin is soluble in absolute methanol. Undissected pituitaries are extracted with water at pH 8–9 and the prolactin isoelectrically precipitated in the presence of chloroform whereupon a chloroform gel is formed containing the prolactin. The latter is extracted from the dried gel with acid methanol and then precipitated with ether at pH 3. Further purification is effected by precipitation with NaCl from aqueous solution at pH 2.5 followed by solution in methanol and precipitation with ether. The yield is 2 g. per kg. of fresh sheep gland, but cattle and hog glands give lower yields of a product not quite so potent. The degree of contamination with other hormones is, at the time of writing, unknown.

The first claim to have prepared crystalline prolactin was made by White, Catchpole and Long (1937), the amorphous starting material being made by the acid-acetone method of Lyons (1937a). The crystals exhibited an activity of the same order as that given by Lyons (1937a) for his purified amorphous preparations and from this fact and other subsequent chemical evidence it seems that Lyons had obtained practically pure prolactin as early as 1936–37. Later, White, Bonsnes and Long (1942) (*see also* White, 1943) described two procedures for obtaining crystalline prolactin in low yield, starting from an acid-acetone preparation which had been carefully separated from adrenocorticotrophin. Crystallisation of the hormone was achieved by an acetic acid-pyridine procedure, as previously described by White *et al.* (1937), or by slow precipitation from saturated aqueous solutions by addition of acetone. The crop-stimulating activity of the crystalline protein agreed well with those of the most active amorphous preparations (30–35 i.u. per mg.) and physico-chemical studies indicated that the preparation was molecularly homogeneous.

Chemistry of prolactin.—Prolactin (pigeon crop-stimulating) activity is associated with a protein which has been prepared in amorphous and crystalline forms which are molecularly homogenous by all physico-chemical criteria at present available. At the present time there is no reason to believe that the actual hormone is an impurity inseparable from the protein. Neither is there any evidence that the biological activity is due to the presence of a prosthetic group since no such group has been detected spectroscopically (*see* White and Lavin, 1940) or by any other means.

The protein nature of prolactin was long ago suspected on account of its solubility relations (*see* Bates, Riddle and Lahr, 1934; Riddle and Bates, 1939). It is soluble in water except in the isoelectric region (pH 5–6). On the basis of electrophoretic studies the following values for the isoelectric point have been given; for crystalline prolactin, pH 5.6 (Shipley, Stern and White, 1939), pH 5.65–5.70 (White *et al.* 1942); for pure amorphous prolactin, pH 5.7 (Li *et al.* 1940a); pH 5.73 (Li *et al.* 1940b). The latter workers found identical values for cattle and sheep hormones. At pH values acid to the isoelectric point the hormone is precipitated by the usual protein precipitants (*see* Riddle and Bates, 1939), and at neutrality by the hydroxides of Zn, Cu, Al, and Fe (Bates and Riddle, 1938).

Figures for the elementary analysis of various crystalline and amorphous preparations from three laboratories are tabulated by White *et al.* (1942); with the exception of the figures for sulphur the various analyses show good agreement. The following percentage figures for crystalline prolactin, from the Table given by White *et al.*, are typical: C = 51.81, H = 6.81, N = 16.49, S = 2.03, ash = 0.50. Tests for phosphorus and carbohydrate were negative. Sulphydryl groups appear to be absent (Li, Lyons and Evans, 1941b; White *et al.* 1942; Fraenkel-Conrat, Simpson and Evans, 1942; Fraenkel-Conrat, 1942) but appear on prolonged reduction with NaCN (White *et al.* 1942) or on treatment with thiol compounds (Fraenkel-Conrat, 1942).

Crystalline and purified amorphous preparations give a protein biuret colour and the usual colour reactions for amino acids (White *et al.* 1942). A broad band in the ultra-violet spectrum of prolactin was attributed by White and Lavin (1940) to the combined absorption of tyrosine, phenylalanine and tryptophane. The tyrosine content of bovine prolactin has been given as 5.73 per cent. (Li, Lyons and Evans, 1940c), 5.42 per cent. (Li, Lyons and Evans, 1941c) and 5.51 per cent. (White *et al.* 1942); somewhat lower values, 4.53 per cent. and 4.7 per cent., have been found for sheep prolactin (Li *et al.* 1940c, 1941c). The following values for the tryptophane content have been given: bovine hormone 1.31 per cent., sheep hormone 1.19 per cent. (Li *et al.* 1940c); bovine hormone, 1.30 per cent. (White *et al.* 1942). Later, Li *et al.* (1941c), using a method considered superior to the one they used formerly, obtained a value of 2.5 per cent for hormone from both sources. The arginine content of sheep and bovine hormones has been given as 8.31 per cent. (Li *et al.* 1941c) and the cystine content as 3.0 per cent. (Fraenkel-Conrat, 1942) and 3.11 per cent. (Li, 1943). No cysteine has been found in prolactin (Li *et al.* 1941b; Fraenkel-Conrat, 1942). Methionine is present to the extent of 4.3 per cent. (Li, 1943), this together with cystine accounting for the total sulphur.

The molecular homogeneity of highly active amorphous or crystalline preparations has been demonstrated by electrophoretic (Li *et al.* 1940a; White *et al.* 1942), solubility (Li *et al.* 1941a; White *et al.* 1942) and ultracentrifugal (White *et al.* 1942) studies. Li *et al.* (1941a) observed differences in the solubilities of sheep and bovine hormones which pointed to species specificity. As already mentioned, however, no such species differences in electrophoretic mobility were observed, nor are there any as regards specific rotation (Li, 1942) while Bischoff and Lyons (1939) found that the hormones from the two sources were immunologically indistinguishable.

At the time of writing the molecular weight of prolactin is not known with certainty. From osmotic pressure determinations Li *et al.* (1941c) obtained a mean value of 26,500 in good agreement with the mean value, 25,000, estimated from the percentages of tryptophane, tyrosine, cystine, arginine and sulphur. On the other hand, determination of the diffusion constant and sedimentation velocity in the ultracentrifuge indicated a molecular weight of 32,000–35,000 on the assumption of a spherical molecule (White *et al.* 1942). Li (1942), however, on the basis of viscosity data, contends that the prolactin molecule is not spherical (dissymmetry constant = 1.29) and from the latter and the diffusion constant estimates the molecular weight as 22,000.

The effect of heat on the biological activity of prolactin depends on conditions

such as pH , the presence or absence of salts and so forth, as has been emphasised by Riddle and Bates (1939). At pH 8 it withstands boiling for one hour except in the presence of salts but is rapidly inactivated by heat at acid or alkaline pH values (Riddle and Bates, 1939). Relatively crude preparations studied by McShan and French (1937) were fairly stable to heat between pH 3.2 and pH 9.7. White *et al.* (1942) reported that pure prolactin in dilute solution undergoes some inactivation after 30 minutes on a boiling water-bath at all pH values between pH 1 and pH 13. The assay technique used (the intradermal minimal response method) was, however, hardly adequate to provide a true quantitative picture of the effects involved, but it is probably justifiable to conclude that pure prolactin is a heat-labile protein. The activity is also destroyed on hydrolysis with acid (White *et al.* 1942), pepsin (McShan and French, 1937; White *et al.* 1942) and trypsin (Bates *et al.* 1934; McShan and French, 1937; White *et al.* 1942). The results of White *et al.* (1942) indicate that the biological activity is destroyed before the disappearance of all protein fragments which can be precipitated by trichloroacetic acid. The presence of free amino groups (probably the ϵ -amino groups of lysine) appears to be necessary for biological activity, since the latter is completely or largely destroyed by treatment with ketene (Li, Simpson and Evans, 1939), nitrous acid (Li, Lyons, Simpson and Evans, 1939) and phenyl isocyanate (Bottomley and Folley, 1940). The biological activity also seems to be bound up with the tyrosine residues since Li *et al.* (1941b) have shown that iodination of prolactin, under conditions in which the iodine appears to combine only with the tyrosine molecules, also decreases its activity. Treatment with a large excess of cysteine (or thioglycollic acid) also causes inactivation of the hormone (Fraenkel-Conrat *et al.* 1942) accompanied, according to Fraenkel-Conrat (1942), by the appearance of SH and other reducing groups. Small proportions of cysteine render the hormone insoluble without destroying its activity (Fraenkel-Conrat *et al.* 1942).

The prolactin molecule appears to be relatively stable since biological activity is retained after treatment with urea (Li *et al.* 1941b) and precipitation with trichloroacetic acid (Li *et al.* 1941c), both of which denature proteins. Osmotic pressure determinations indicate that this molecule, unlike those of many proteins, does not fragment in presence of urea (Li *et al.* 1941c). It is of course possible that the hormone may be reversibly denatured by urea without undergoing a change in molecular weight.

It is clear that considerable information has already been accumulated with regard to the chemical, and particularly the physico-chemical, properties of the prolactin molecule. Moreover, a beginning has been made with the correlation of chemical structure with biological activity. It must be emphasised, however, that in many of the inactivation experiments discussed above, the assay methods, which in some cases amounted to little more than qualitative tests and subjective ones at that, have not been adequate for quantitative studies. Further work with amounts of material allowing accurate assay of reaction products in terms of international units, preferably by means of the objective crop-weight method, may be expected to yield additional information on quantitative aspects.

Physiological responses to prolactin.—In addition to the pigeon crop-gland response, already dealt with, other physiological mechanisms not primarily concerned with lactation seem to involve prolactin (for review *see* Riddle, 1937). The rôle of this hormone in lactation, undoubtedly its best-known physiological

function, has already been considered and will be further discussed in later sections, but the presence of prolactin in non-mammalian forms indicates that lactation cannot be the only, or necessarily the most important, physiological function in which it is involved.

There is now reason for the belief that prolactin must be considered to be a member of the anterior-pituitary gonadotrophic complex. An antigonadotrophic action of prolactin was first described by Riddle and Bates (1933) who found that prolactin decreases the testis weight in male pigeons. Later (Bates, Lahr and Riddle, 1935), it was found that the ovary (and secondarily the oviduct) of the domestic hen responded similarly. Such effects were thought to be due to interference with the secretion or release of F.S.H. by the pituitary since simultaneous treatment with F.S.H. abolished the effect of prolactin on the pigeon testis (Bates, Riddle and Lahr, 1937). Prolactin is also capable of influencing, directly or indirectly, the ovaries of mammals. Thus treatment with prolactin interrupts oestrous cycles in the mouse (Dresel, 1935) and rat (Lahr and Riddle, 1936; Lyons, Simpson and Evans, 1941), but as yet has not been found to affect the length of the menstrual cycle in the monkey (Hisaw, 1944) and woman (Kupperman, Fried and Hair, 1944). Lyons *et al.* (1941) contended that since prolactin did not delay vaginal opening in the rat it probably does not directly suppress the output of F.S.H. Persisting vaginal mucification and development of mammary lobule-alveolar tissue in rats in prolactin anoestrus, pointed to the continued secretion of progesterone by the persistent corpora lutea. In their experiments Lahr and Riddle (1936) had also obtained histological evidence of continued luteal function under the influence of prolactin.

Subsequently, further evidence of the luteotrophic action of prolactin has accumulated. Astwood (1941) postulated the existence of a new pituitary gonadotrophin, luteotrophin, responsible for the control of luteal function and present in extracts prepared by methods similar to those used for prolactin. Using the formation of traumatic uterine placentomata as an index of luteal function, Evans, Simpson and Lyons (1941) and Evans, Simpson, Lyons and Turpeinen (1941) have shown that prolactin is probably the only pituitary hormone which regularly maintains luteal function in hypophysectomised rats. Further evidence, based on the deciduoma response, of the luteotrophic action of prolactin has been presented by Lyon (1942), Fluhman and Laqueur (1943) and Sydnor (1945), while Cutuly (1941a, b; 1942) has interpreted his observations that prolactin tends to favour implantation of ova and to some extent maintain pregnancy in rats hypophysectomised soon after mating, as evidence that prolactin exerts a luteotrophic action. Additional evidence comes from the work of Nelson and Pichette (1943) who found that prolactin will prevent the return of oestrus which ordinarily follows hypophysectomy in rats in which pseudo-pregnancy has been induced and maintained by oestrogen, and of Everett (1944) who showed that besides progesterone, prolactin (in the presence of luteal tissue) would interrupt continuous oestrus in rats of a strain prone to exhibit this condition under constant illumination. Moreover, it should not be forgotten, that Tobin (1942) has found that certain prolactin preparations will, like progesterone itself, prolong the survival of adrenalectomised rats.

Riddle and Bates (1939) have pointed out that prolactin is particularly concerned in responses related to the care of the young. It has been said to induce

maternal behaviour in rats (Riddle, Lahr and Bates, 1935), under certain conditions broodiness in hens (Riddle, Bates and Lahr, 1935), and nesting behaviour in fish (Noble, Kumpf and Billings, 1936).

Various metabolic effects of prolactin have been described but it seems doubtful whether these are specific and primary responses to prolactin. According to Bates, Riddle, Lahr and Schooley (1937) prolactin treatment increases the weights of the liver and intestine (and also the length of the latter) in the hypophysectomised pigeon. Prolactin has also been said to stimulate general body growth in the normal and hypophysectomised pigeon (*see* Riddle and Bates, 1939) and, in conjunction with thyrotrophin, in genetically dwarf mice (Bates, Laanes and Riddle, 1935). A calorogenic action of prolactin has been reported in the dove in the absence of the adrenals (Riddle and Smith, 1940) and the thyroid (Riddle, Smith, Bates, Moran and Lahr, 1936). Evidence about the effects of prolactin on carbohydrate metabolism has been rather conflicting. Preparations used by Nelson, Turner and Overholser (1935) had no effect on the blood sugar of mammals but Riddle, Dotti and Smith (1937) reported hyperglycaemia in response to prolactin in pigeons. Equally conflicting are reports of the effect of prolactin on the insulin content of the pancreas. According to Funk, Chamelin, Wagreich and Harrow (1941) prolactin decreases the pancreatic insulin; the reverse is claimed by Fraenkel-Conrat, Herring, Simpson and Evans (1942, 1944). According to Gaebler and Robinson (1941, 1942) prolactin, in contradistinction to other anterior-pituitary fractions, caused nitrogen storage in depancreatized bitches kept on a constant insulin dosage. Under these conditions the calorogenic and diabetogenic effects of the hormone were relatively mild.

Occurrence of prolactin.—Prolactin has been found in anterior-pituitary tissue from all mammals so far examined, including man. The available evidence points to the eosinophiles as the site of synthesis of the hormone (Schooley and Riddle, 1938; Azimov and Altman, 1938; Friedman and Hall, 1941). Pituitaries from the sheep, the ox, and to a lesser extent, the pig, provide the principal sources for large-scale preparation. According to Lyons (1937b) posterior lobes also contain considerable amounts of prolactin, probably because of *post-mortem* diffusion. As regards sub-mammalian classes, positive responses by the local crop test have been obtained with pituitaries of birds (Burrows and Byerly, 1936; Leblond and Noble, 1937), reptiles, amphibia and fish (Leblond and Noble, 1937). The prolactin content of the mammalian pituitary under various conditions will be considered in the next section.

Positive crop reactions have also been obtained with liver tissue (or extracts thereof) from various species (Leblond and Noble, 1937; Keller, 1939; Lessmann, 1939; Rabald and Voss, 1939; Cunningham, Bickell and Tanner, 1940), with placental (particularly unripe) tissue (Ehrhardt, 1936; Keller, 1939; Turner and Meites, 1941a) and mammary gland (Geschickter and Lewis, 1936). Positive intradermal crop tests must at best be considered as tenuous evidence of the occurrence of prolactin in tissues other than mammalian anterior lobe in view of the non-specific crop responses described by Lahr *et al.* (1943) (*see also* Leblond and Noble, 1937), but it seems possible that the liver responses may be actually due to prolactin since Rabald and Voss (1939) obtained an active liver extract, by essentially the method of Bates and Riddle (1935), which was assayed by a crop-weight method. The presence of a crop-stimulating substance in adrenal cortex

extracts was claimed by Hartman, Lockwood and Brownell (1933). Later, the active substance, called cortilactin, was separated by isoelectric precipitation at pH 5.8 but no claim as to its identity with prolactin was made (Spoor, Hartman and Brownell, 1941). Hurst, Meites and Turner (1942), on the other hand, failed to detect crop-stimulating material in adrenal cortex extracts.

Crop-stimulating substances have been detected in blood from the turtle (Cunningham *et al.* 1940), the rabbit (Meites and Turner, 1942a), the lactating cow and mare (Leblond, 1937) and woman (Tesauro, 1936; Ehrhardt and Voller, 1939). The latter workers reported peak titres at ovulation and menstruation.

The first detection of prolactin in *post-partum* human urine was achieved by Lyons and Page (1935) and later, assays were carried out by Tesauro (1936), Hoffman (1936), Lessmann (1939), Ehrhardt and Voller (1939) and Meites and Turner (1941). Prolactin has also been detected in the urine of men (Lyons, 1937b) and in urine of new-born infants of both sexes (Lyons, 1937c). Hoffmann (1936) found no prolactin in human urine before parturition and reduced excretion in cases of hypogalactia (*see also* Meites and Turner, 1941). Hurst, Meites and Turner (1941) reported positive responses with urine from lactating goats. Positive mammalian lactogenic responses with human urine extracts were reported by Langecker and Schenk (1936) and Liard (1937).

In general comment on the foregoing results it may be well to re-emphasise that as regards tissues other than mammalian pituitary tissue it is not safe to deduce the presence of prolactin on the sole evidence of positive crop tests. The only sure criterion would be the isolation of a pure protein and its chemical and biological characterisation. Further attempts to isolate pure prolactin from mammalian liver would therefore be of interest. The occurrence of prolactin in the blood and urine of mammals, at any rate during lactation, is *a priori* to be expected and it seems likely that the crop-stimulating reactions obtained with these body fluids are in fact due to prolactin.

Prolactin content of the pituitary gland.—Species differences in the prolactin content of the pituitary gland were reported by Reece and Turner (1937b) who, using a modification of the intradermal crop test, arranged the pituitaries from females of various species in descending order of potency as follows: ox (dairy breed), guinea-pig, rat, rabbit, mouse, cat. The results for males were in harmony with these. Later, Holst and Turner (1939a) obtained a similar ranking for pituitaries of female guinea-pigs, rats and rabbits. Chance, Rowlands and Young (1939) assayed, by a crop-weight method, partially purified extracts of batches of glands and gave the following descending order of potency for sources which might be useful for large-scale preparation: sheep, ox, man, pig, horse. Bates and Riddle (1935) had already found that pig glands were much less potent than those of the sheep or ox.

The existence of strain or breed differences within a species, particularly as between cattle of dairy and beef breeds respectively, might be expected and indeed Reece and Turner (1937b) claim that glands from dairy cows contain more hormone than glands from beef cows. Hall (1944b), however, criticises the method of assay used by Reece and Turner (1937b), which involved the injection of material in suspension, since he found that extracts gave higher results than suspensions. With Hall's technique there was no difference in prolactin content between pituitaries from dairy and beef cattle (*see also* Hall and Nicolet, 1942). The

work of Hall raises the important issue of the validity of many of the rather fine differences in the prolactin content of the pituitaries of small animals, to be discussed below, since the assays involved the intradermal injection of tissue suspensions.

As regards sex differences, Bates, Riddle and Lahr (1935) could find no marked differences between the potencies of anterior-lobe tissue from non-pregnant cows and bulls (or steers) while, on the other hand, among a variety of species Reece and Turner (1937b) and Holst and Turner (1939a) generally found female pituitaries more potent than those from males. It may be noted that Bates *et al.* assayed partially purified extracts by a crop-weight method while Turner *et al.* used the local crop test.

Considerable differences in the prolactin content of the pituitary, in both sexes at different stages of maturity and in different reproductive states, have been reported. Of seven classes of cattle pituitaries assayed by Bates *et al.* (1935) foetal pituitaries were the most potent; by contrast, Reece and Turner (1937b) found less prolactin in foetal pituitaries than in any other type of cattle pituitary examined. In the bull, Reece and Turner (1937b) reported an increase, with advancing maturity, in the total prolactin per gland, mainly due to increasing pituitary size. An increase in the pituitary prolactin in the male guinea-pig at puberty was noted by Holst and Turner (1939a). The results of Bates *et al.* (1935) and Reece and Turner (1937b) were once more in conflict, this time about the effect of castration on the prolactin content of the bull pituitary, the results of the former indicating that castration has little effect while the latter reported a significant decrease following the operation. In the male rat, however, Reece and Turner (1937b) found no change in pituitary prolactin after castration or experimentally induced cryptorchidism.

According to Turner and his colleagues, sexual maturity in the female is accompanied by an increase in the prolactin content of pituitary tissue (Reece and Turner, 1937b; Holst and Turner, 1939a). Increased values in the rat have been reported at oestrus and conversely, decreased values following ovariectomy (Reece and Turner, 1937b). In the mouse, however, ovariectomy did not affect the pituitary prolactin (Hurst and Turner, 1942). Some workers have reported increased values during pregnancy (*see* Bates *et al.* 1935 and Reece and Turner, 1937b, for the cow; Wiegand, 1937, for the rat; Hall and Nicolet, 1942, for the rabbit; Hurst and Turner, 1942, for the mouse), but others report no increase during pregnancy in the rat (Reece and Turner, 1937b), guinea-pig (Holst and Turner, 1939b) and rabbit (Turner and Meites, 1941a; Holst and Turner, 1939b). Similarly conflicting results have been reported concerning the effect of pseudo-pregnancy in the rabbit; Hall and Nicolet (1942) reported increased assays during pseudo-pregnancy while Meites and Turner (1942b) could find no such effect.

Turner and his school have put forward considerable evidence that parturition is followed by a rise in pituitary prolactin potency which rise they associate with the initiation of lactation (*see* later). Data have been obtained on the mouse (Hurst and Turner, 1942), rat (Reece and Turner, 1937b), guinea-pig (Reece and Turner, 1937b; Reece, 1939) and rabbit (Reece and Turner, 1937b; Holst and Turner, 1939b; Meites and Turner, 1942f). Confirmatory data for the rat were given by Wiegand (1937). Assays of the prolactin content of the pituitary at different stages of lactation have been made by Hurst and Turner (1942) in the

mouse, Reece, Hathaway and Davis (1939) in the rat and Meites and Turner (1942f) in the rabbit. In general, the values show a progressive decline but it is thought that the maximum attained does not coincide with the peak of lactation.

In a later section evidence will be considered which indicates that suckling, probably through a nervous reflex evoking the discharge of hormones by the anterior pituitary, may affect the function of the mammary glands. It is therefore of some interest to note that Reece and Turner (1936b, 1937a, b) found that, in the rat, the suckling stimulus following a period of non-suckling caused a decrease in the pituitary prolactin content (*see also* Holst and Turner, 1939b, for similar results in the guinea-pig and rabbit). Later, Meites and Turner (1942c, f) presented evidence that regular application of the suckling stimulus is capable of exerting a long-term effect on the prolactin content of the pituitary; they found less prolactin in the pituitaries of parturient rats and rabbits prevented from suckling than in normally nursing controls. It should be noted, however, that Meites, Bergman and Turner (1941b) found that in the rabbit the size of the litter suckled had no effect on the pituitary prolactin.

The effect of oestrogen on the prolactin content of the pituitary is of interest in view of current ideas about the rôle of oestrogen in lactogenesis and galactopoiesis (*see later*). All the available evidence indicates that by contrast with the known suppressive effects of oestrogen on the secretion of F.S.H. and probably also growth hormone by the pituitary, the secretion of prolactin is increased (for cytological evidence of hypersecretion by the anterior pituitary under oestrogen influence, *see* Zeckwer, 1944). Reece and Turner (1936a) first reported an increase in the pituitary prolactin in the male rat under the influence of oestrogen. This has since been repeatedly confirmed in rats, rabbits and guinea-pigs (Reece and Turner, 1937b; Reece, 1938; Lewis and Turner, 1941c; Meites and Turner, 1942a, c) and according to Meites and Turner (1942c) occurs in lactating rats despite the fact that oestrogen tends to inhibit established lactation. Wiegand (1937), however, found less prolactin in the pituitaries of parturient rats given sufficient oestrogen to inhibit the growth of the young than in those of controls. It is of course possible that if the observed increases in pituitary prolactin content following oestrogen administration are real, they merely signify an inhibition of release rather than increased secretion. Meites and Turner (1942a), however, using the intradermal crop test, reported an increase in the blood prolactin of oestrogenised rabbits coinciding with the increased potency of the pituitary, which would indicate an actual increase in prolactin production. The data, however, are hardly extensive enough to prove such an important point beyond doubt.

Turner and his colleagues have found that progesterone by itself does not increase the prolactin content of the pituitary (Reece and Turner, 1937b; Meites and Turner, 1942d) but given in combination with oestrogen tends to inhibit the effect of the latter (Meites and Turner, 1942d). Hence they believe that the corpus luteum prevents a rise in the pituitary prolactin during pregnancy. In keeping with this view they reported an increase in the prolactin content of the pituitary of the pregnant rabbit following hysterectomy (Meites and Turner, 1942e). Reece and Bivins (1942), however, found that high doses of progesterone (15 mg. daily) increased the prolactin content of the pituitary in the spayed female rat. Testosterone has been reported to increase the pituitary prolactin (Reece and

Mixner, 1939 ; Meites and Turner, 1942c) but androsterone (200 μ g. daily) was inactive (Reece, 1941). Meites, Trentin and Turner (1942) reported that the pituitary prolactin in the rat was reduced by adrenalectomy, though the *post-partum* increase still occurred. Deoxycorticosterone, however, did not affect the pituitary of the mouse (Turner and Meites, 1941b).

McQueen-Williams (1935), who introduced the widely used technique for the assay of the prolactin content of the rat pituitary, reported decreased values in thyroidectomised rats. Reineke, Bergman and Turner (1941), on the other hand, found no such effect in young male goats ; neither was the prolactin content of the mouse pituitary altered by the administration of thyroxine (Hurst and Turner, 1942).

Lactogenic action of anterior-pituitary hormones in hypophysectomised animals.— It is now commonly accepted that hypophysectomised test animals should be used for the investigation of primary responses to anterior-lobe hormones in order to eliminate possible disturbing factors due to the action of endogenous pituitary hormones. If lactogenic hormones are defined as anterior-pituitary hormones which initiate lactation in *hypophysectomised* animals, the generally accepted theory (see for example Bergman and Turner, 1940 ; Lyons, 1941) of the existence of a single lactogenic hormone, prolactin, cannot be maintained, since it has generally been found that purified prolactin will not initiate lactation in hypophysectomised animals possessing mammary glands which might be expected to respond in presence of the pituitary (Gomez and Turner, 1936b, 1937c ; Nelson and Gaunt, 1936, 1937a ; Nelson *et al.* 1943). The work of Fredrikson (1939) provides an exception since he reported initiation of lactation in hypophysectomised rabbits with purified prolactin. Unfractionated anterior-pituitary preparations, on the other hand, have been generally found to initiate lactation in hypophysectomised animals (see McPhail, 1935a, b, for the ferret and cat ; Houssay, 1935b, for the dog ; Gomez and Turner, 1936b, 1937c and Nelson and Gaunt, 1936, 1937b, for the guinea pig). From these results it may be concluded that if, as appears probable on the basis of experiments with intact animals, prolactin is concerned with lactogenesis, it is not the only anterior-pituitary hormone so concerned.

There is considerable evidence that adrenocorticotrophin is also involved in lactogenesis since lactation can be initiated in hypophysectomised guinea pigs by treatment with purified prolactin (and sometimes glucose) together with adrenal cortex extract (Gomez and Turner, 1936d, 1937c ; Nelson and Gaunt, 1936, 1937b), certain cortical steroids (Nelson *et al.* 1943) or adrenotrophin (Gomez and Turner, 1937a ; Nelson and Gaunt, 1937a). Gomez and Turner (1937b, c) could obtain no evidence of the importance of thyrotrophin in this connection, since combinations of prolactin and thyroxine were ineffective.

Folley and Young (1941a), in a discussion of the status of prolactin as a lactogenic hormone, pointed out that if these results are to be relied upon—and admittedly in view of Fredrikson's (1939) positive results in rabbits the existing rather sparse data on lactogenesis in the absence of the hypophysis should be extended by experiments on a variety of species with the purified pituitary hormones now available—adrenotrophin must be conceded an equal claim with prolactin to be regarded as a lactogenic hormone since either may be considered capable of initiating lactation in the presence of the other. That the two hormones may play quite distinct

rôles in lactogenesis, prolactin perhaps being necessary for the expression of the synthetic activities of the alveolar cells and adrenotrophin probably influencing through the adrenal cortex the supply of milk precursors, does not affect the point at issue. Thus, in place of the conventional single lactogenic hormone theory, Folley and Young (1941a) were led to the conception of a lactogenic complex of anterior-pituitary hormones, two members of which, prolactin and adrenotrophin, seem already to have been identified. Others probably remain to be discovered since the purified preparations used in the aforementioned experiments doubtless contained other unidentified hormones.

Bergman *et al.* (1940) have affirmed their belief in the existence of a single anterior-pituitary lactogenic hormone, prolactin, since they assayed a prolactin preparation at different stages of purification by methods depending on the pigeon crop-gland and mammalian lactogenic responses and found that purification did not alter the ratio of the potencies of the extract expressed in the two types of unit. This might merely mean, however, that components of a lactogenic complex were concentrated in the same proportions. As further evidence in favour of the single lactogenic hormone theory, Bergman and Turner (1940) have cited the ability of the prolactin moiety of a fractionated anterior-pituitary extract regularly to initiate lactation in suitably prepared rabbits as against the comparative failure to do so of the remaining fraction containing certain other hormones but little prolactin. Among other criticisms of this paper of Bergman and Turner, Folley and Young (1941a) pointed out that since rabbits with intact pituitaries were used, their results, which undoubtedly indicate that prolactin can be a limiting and essential factor in lactogenesis, are hardly relevant to the question of the existence or otherwise of a single lactogenic hormone. More recently, Turner (*see* Hurst and Turner, 1942) appears to have accepted the view that more than one hormone is concerned with the initiation of lactation since in the mouse the prolactin content of the pituitary, a rise in which is regarded by Turner and his school (*see* Meites and Turner, 1942d) as a decisive factor in the initiation of lactation, increases considerably during pregnancy though no milk secretion occurs.

The action of prolactin on the mammary gland has generally been assumed to be direct and this assumption has been regarded as in accord with the theory that prolactin is the specific lactogenic hormone. Thus Bergman *et al.* (1940) apparently envisaged the action of prolactin on the mammary gland as in the nature of a "trigger" effect, since lactation was assumed to follow the "activation" of the alveolar cells by prolactin. It is only recently, however, that any proof of the direct action of prolactin has been provided. Lyons (1942) essayed to obtain such proof by elegant experiments in which minute amounts of prolactin were introduced into individual galactophores of the rabbit mammary gland. Lactation (and, according to Lyons, epithelial hyperplasia) was induced in those gland sectors communicating with the injected galactophores but not in adjacent sectors (*see* Fig. 20. 13.). But the rabbits were not hypophysectomised, and while these results point to the possibility of a direct effect on the mammary epithelium they do not demonstrate that prolactin is the only hormone concerned in lactogenesis, since the co-operative action of endogenous pituitary hormones was not excluded.

Galactopoietic action of anterior-pituitary extracts.—In the present state of knowledge it is desirable, for clear discussion, to adopt different terms in respect of the initiation of lactation and its stimulation once established. Folley and Young

(1940) suggested that as long as it should be thought necessary to draw a distinction between these two processes they should be respectively designated by the terms *lactogenesis* and *galactopoiesis*. Bergman and Turner (1940) subsequently and independently suggested a similar terminology.

Following the discovery of the lactogenic properties of anterior-pituitary extracts, galactopoietic effects of the crude extracts then available were soon demonstrated in farm animals (*see* Grüter and Stricker, 1929, for the cow and sow ; Asdell, 1932, for the goat ; Kabak and Kisilstein, 1934, and Kabak and Margulis, 1935, for the sheep). With the discovery of prolactin and its characterisation as a hormone concerned in lactogenesis arose a belief that prolactin would possess outstanding galactopoietic properties which might find application both in

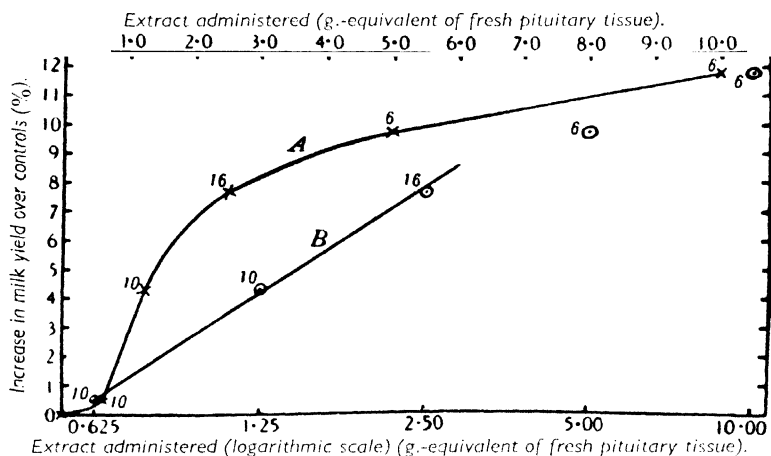


Fig. 20. 15—The influence of a single injection of ox anterior-pituitary extract on the milk yield of groups of cows in declining lactation. The figures attached to the points give the number of cows in the group. Curve A shows the mean daily increase in milk yield over the two days following the injection plotted against the dose of extract given. Curve B is a plot of the responses against the logarithm of the dose of extract given. (From Folley and Young, 1945.)

medicine and in the dairy industry. This idea has, however, not been substantiated by subsequent work. It was first reported by Azimov and Krouze (1937) and later shown more conclusively by Folley and Young (1938) that on the basis of equal prolactin content, determined by pigeon crop methods, unfractionated anterior-pituitary extracts exhibit much more galactopoietic activity, assayed in lactating cows, than purified prolactin. Both groups of workers reported substantial, temporary increases in milk yield following single injections of crude ox anterior-pituitary extracts into lactating cows (*see* Fig. 20. 15), but whereas Azimov and Krouze (1937), whose paper should be consulted for references to earlier papers on the subject by Azimov and his colleagues, found the treatment to be most effective during the first four months of lactation during which time the yield could be increased slightly above the natural peak value, Folley and Young (1938, 1940, 1945) obtained good responses during the declining phase, and subsequently showed that their crude saline extract was ineffective at the time of peak milk yield (Fawns, Folley and Young, 1945). Similarly in goats

Asdell *et al.* (1936) found that anterior-pituitary extracts were effective during the declining phase but not at the peak of lactation. Folley and Young (1945) obtained a sigmoid dose-response curve for single injections of crude ox anterior-pituitary extract in cows, and later showed that horse gland extracts were even more active than extracts of ox pituitary while material from the sheep and pig possessed little activity (Folley *et al.* 1945b).

In contrast to the relative ineffectiveness of single injections of purified prolactin, repeated injections of relatively high doses of prolactin preparations produced substantial increases in milk yield (Folley and Young, 1938, 1939, 1940). In some experiments the initial stimulus soon disappeared despite continuance of the treatment, but this did not appear to be due to the formation of anti-prolactin (Folley and Young, 1940).

As with single injections, the galactopoietic effect of repeated injections of crude ox pituitary extracts was, on the basis of equal prolactin content, considerably greater than that of prolactin fractions (Folley and Young, 1940), but repeated injections of crude extracts of sheep and pig gland actually decreased the milk yields of lactating cows and here it seems possible that the formation of anti-hormones to heterologous glandular material was involved (Folley *et al.* 1945b). Various technical factors connected with the practical use of crude extracts of ox pituitary for increasing the milk yield of cows were studied by Folley and Young (1945).

Thyrotrophic preparations from ox anterior lobe, containing little or no prolactin, also exhibited galactopoietic activity in cows (Folley and Young, 1938, 1939) but it is not certain that these responses were mediated by the thyroid since no rise in milk fat content, a characteristic result of thyroxine treatment (*see* page 584), occurred.

Changes in milk composition in response to pituitary treatment seem in general to be slight (Azimov and Krouze, 1937; Folley and Young, 1938), but marked increases in lactose (Folley and Young, 1938; Sykes, Gould, Duncan and Huffman, 1944) and fat (Folley and Young, 1939; Sykes, Mueleman and Huffman, 1942) contents have been observed in response to certain extracts.

Folley and Young (1938, 1939, 1940) investigated the relation between galactopoietic activity and certain other biological properties of a number of anterior-pituitary preparations. Galactopoietic activity bore little relation to prolactin content but was closely paralleled by glycotropic (anti-insulin) activity, save that an extract rich in glycotropin but free from prolactin showed no galactopoietic activity (Folley and Young, 1940). Similarly, the galactopoietic activities of anterior-pituitary extracts from four species (Folley *et al.* 1945b) bore no relation to their relative prolactin contents (Chance *et al.* 1939). It was concluded that, like lactogenesis, galactopoiesis must be ascribed to the action, not of a single anterior-pituitary hormone, but rather of a hormone complex of which prolactin and glycotropin are members. A claim to have identified glycotropic action with adrenotrophin has been made by Jensen and Grattan (1940). Folley and Young (1941a) pointed out that if this idea should prove to be correct, the necessity for drawing a distinction between lactogenic and galactopoietic activities, as far as anterior-pituitary extracts are concerned, would be open to doubt since the same combination of hormones would have been found to play an important rôle in both processes.

Purified prolactin preparations have been used for the treatment of deficient lactation in women with conflicting and sometimes disappointing results (Kurzrok, Bates, Riddle and Miller, 1934 ; Ehrhardt, 1936 ; Hoffmann, 1936 ; Ross, 1938 ; Lessmann, 1939 ; Stewart and Pratt, 1939 ; Werner, 1939). The most successful results seem to have been obtained by Kenny and King (1939) and Winsor (1943). Folley and Young (1941a) point out that lack of agreement about the clinical efficiency of prolactin is not surprising since the extracts used have been assayed only for prolactin content on the tacit assumption that this is a measure of galactopoietic power. The lactating woman is surely more analogous, from the physiological point of view, to the lactating cow than to the pigeon and it would therefore seem desirable to use for clinical purposes, preparations which have been assayed for galactopoietic potency in lactating mammals.

Oestrogen

Inhibitory effect of oestrogen.—It has been generally believed for many years that oestrogens exert an inhibitory effect on lactation. The first direct evidence of such an effect was provided by Parkes and Bellerby (1927) who showed that administration of oestrogenic extracts to lactating mice depressed the growth of their young. With the advent of crystalline and synthetic oestrogens confirmatory evidence has been provided in the mouse (de Jongh, 1933a ; Robson, 1935), rat (de Jongh, 1933b ; Wade and Doisy, 1935 ; Anselmino and Hoffmann, 1936 ; Folley and Kon, 1937 ; Folley and Scott Watson, 1938 ; Noble, 1939a ; Reece, Bartlett, Hathaway and Davis, 1940 ; Edelmänn and Gaunt, 1941), guinea-pig (Nelson, 1934a ; Sardi, 1935), rabbit (Smith and Smith, 1933), goat (Mixner *et al.* 1944 ; Folley *et al.* 1945a) and cow (Folley, 1936 ; Waterman, Freud and Vos-de Jongh, 1936 ; Folley, Scott Watson and Bottomley, 1941a ; Stanley and Owen, 1941).

In accord with this conception, while not providing decisive proof of it, are the following facts : (a) administration of chorionic gonadotrophin or pregnant mares' serum has been generally found to inhibit lactation in the presence of the ovary (Enzmann and Pincus, 1933 ; de Jongh, 1933b ; Selye *et al.* 1934 ; Connon, 1937 ; but *see also* Hathaway, Davis, Reece and Bartlett, 1939, and Reece *et al.* 1940, whose results were rather indecisive) but not in its absence (de Jongh and van der Woerd, 1939 ; Edelmänn and Gaunt, 1941), which indicates that ovarian hormones, probably but not certainly oestrogen (*see* Edelmänn and Gaunt, 1941), are involved ; (b) in guinea-pigs complete mammary growth may be induced by oestrogen treatment and lactation sets in when the dose is suddenly decreased (for references *see* page 542) ; (c) grafting or retention of placental tissue inhibits lactation (Frankl, 1923 ; Smith and Smith, 1933 ; but *see* Selye *et al.* 1934, for opposite results).

Oestrogens have been widely used by clinicians for inhibiting lactation when this is desirable. Clinical reports are too numerous to be quoted ; for a recent, well-controlled study *see* Walsh and Stromme (1944).

Some workers have questioned the reality of the inhibiting action of oestrogen on lactation. Anselmino and Hoffmann (1936), for instance, claimed that the effect could not be demonstrated in the absence of the ovary and contended that the agent directly responsible for lactational inhibition was produced by the corpus luteum in response to oestrogen. Progesterone by itself did not appear to

be implicated, however, since purified luteal extracts were ineffective. Folley (1942) has subsequently shown that 15 mg. progesterone daily causes not the slightest lactational inhibition in the rat. Moreover, inhibition by oestrogen in the absence of the ovary has been demonstrated in the mouse (de Jongh, 1933a; Robson, 1935) and rat (Folley and Kon, 1937; Edelman and Gaunt, 1941), significantly higher doses being required in ovariectomised rats than in intact ones. The reality of the inhibitory effect has also been questioned by Reece and Turner (1937b) and Meites and Turner (1942c), by the latter principally because they observed not a decrease but an increase in the prolactin content of the pituitary of the lactating rat in response to high doses of oestrogen. Yet Meites and Turner (1942c) did in fact note evidence of decreased milk secretion in oestrogenised lactating rats which they attributed to suppression of the secretion of anterior-pituitary galactopoietic hormones other than prolactin. They apparently regarded an experimentally produced depression of lactation as a true inhibition only when associated with a decrease in the prolactin content of the pituitary. More recently, however, Turner and his school have accepted the reality of the oestrogen inhibition (Mixner *et al.* 1944) and have speculated on the biochemical mechanisms involved. Scepticism as to the usual interpretation of the effects of oestrogen in puerperal women has been expressed by Abarbanel and Goodfriend (1940). They point out that in women the situation is normally complicated by the absence or removal of the milking stimulus, and they believe that the beneficial effects of oestrogen are entirely due to the prevention of engorgement, a condition due to lymphatic and venous stasis.

It is true that much of the evidence in favour of the inhibition theory was obtained on small animals in which lactational performance can only be indirectly inferred from the growth of the young. Admittedly such experiments should be interpreted with caution since it has been claimed that oestrogen administered to lactating animals may appear in the milk in sufficient amounts directly to affect the growth and well-being of the sucklings (Weichert and Kerrigan, 1942). Nevertheless, the balance of the evidence obtained on farm animals, in which milk yield can be directly measured, would appear to be decisive. Admittedly the dosages necessary to inhibit lactation are almost certainly unphysiological and the importance of oestrogen as a factor preventing lactation in the pregnant animal is thus open to question.

Lactogenic and galactopoietic effects of oestrogen.—Even while the belief was widely held that the effects of oestrogen upon lactation were solely inhibitory, evidence was coming forward which indicated that quite opposite effects were possible under some circumstances. Thus, in the course of studies of experimental mammary growth, some workers had observed evidence of secretion in the experimentally grown glands. As perhaps the most striking instance, may be quoted the results of Frazier and Mu (1935), whose male oestrogen-treated rabbits eventually came into milk and in some cases suckled young.

Results pointing in the same direction were soon afterwards reported by Folley (1936), who observed prolonged increases in the fat and non-fatty solids content of the milk of cows given single injections of oestrogen. This "enrichment effect" represents a true galactopoiesis because in favourable cases there is no more than a transient decrease in yield, and the daily output of milk solids is increased over a considerable period (Folley *et al.* 1941a). Moreover, since the

milk nitrogen partition is unchanged (Folley, 1936) and the lactose content increases (Folley and Scott Watson, 1938; Spielman, Ludwick and Petersen, 1941), there is no question of a change to colostrum such as had been reported by de Fremery (1938) in oestrogen-treated lactating goats.

The most striking evidence of the ability of oestrogens to induce lactation under suitable conditions has been obtained on farm animals. Hormonal induction of lactation in goats, under circumstances in accord with classical views, was long ago achieved by de Fremery (1936, 1938). Udder growth was induced by percutaneous application of an ointment containing oestradiol monobenzoate, but lactation only set in when the oestrogen treatment was replaced by prolactin injections. A revision of classical concepts became necessary, however, when Folley, Scott, Watson and Bottomley (1940, 1941b) showed in the goat that not only mammary growth but also copious and prolonged lactation resulted from treatment (by inunction) with diethylstilboestrol alone. Two points of importance emerged from this work; first, that lactation was initiated in the absence of prolactin treatment and second, that secretion began, and the yield steadily rose to its peak, during the period of oestrogen administration. Thus there was no doubt that a galactopoietic effect of oestrogen, probably mediated by the anterior pituitary, was involved. After the oestrogen treatment was stopped the goats continued to milk for long periods, the lactation curves being similar to those for normal lactation. These results in the goat were generally confirmed by Lewis and Turner (1940, 1941a, 1942a).

Similar experiments by the inunction method in nulliparous heifers were less successful (Folley *et al.* 1941c), only small quantities of colostrum being secreted, but meanwhile Walker and Stanley (1940, 1941) had obtained promising yields from nulliparous heifers by repeated injections of diethylstilboestrol dipropionate, sometimes in conjunction with androgen. Hammond, Jnr., and Day (1944) successfully applied the tablet implantation method to the practical solution of the problem and the extensive investigations on dry cows, nulliparous heifers and freemartins with this technique also include those of Folley and Malpress (1944a), Folley *et al.* (1944) and Spriggs (1945). Oral administration has so far proved less effective (Folley and Malpress, 1944b) but results of some promise were obtained by Parkes and Glover (1944) with a convenient technique involving one injection of a mixture of diethylstilboestrol esters. Consideration of the extensive results so far obtained by the tablet implantation method indicates that some 50 per cent. of the treated animals may be expected to give an economic yield of milk. In a few favourable cases peak yields of over 30 lb. daily and lactation yields of nearly 1000 gallons have been obtained (Fig. 20. 16). A striking feature of the results, and a drawback from the point of view of practical application, is the large and hitherto unexplained individual variation in response.

It seems possible that more uniform responses might be obtained by combined treatment with oestrogen and progesterone since Mixner and Turner (1943) found that in the goat the glandular development induced by such combined treatment was more normal histologically than when only oestrogen was given. Unpublished experiments by Folley and Malpress, in which the effect of treatment of virgin goats with oestrogen and progesterone in various ratios was compared with that of treatment with oestrogen alone, however, provide no evidence of the superiority of the former treatment. Animals with intact ovaries were used in the

majority of the experiments quoted above, but in most cases no functional corpora lutea could have been present in the ovaries during the period of treatment since prolonged oestrogen treatment appears to render the bovine ovary hypoplastic (Folley and Malpress, 1944a; Folley *et al.* 1944; Hammond, Jnr., and Day, 1944).

The chemical composition of the artificially induced secretions has been investigated in the goat by Folley *et al.* (1941b, 1945a), and in the bovine by Folley and Malpress (1944c). At first the secretions resemble colostrum in composition but slowly change to normal milk. In general, a daily yield of 500 ml. in the goat and 5 lb. in the bovine is indicative of normality in composition.

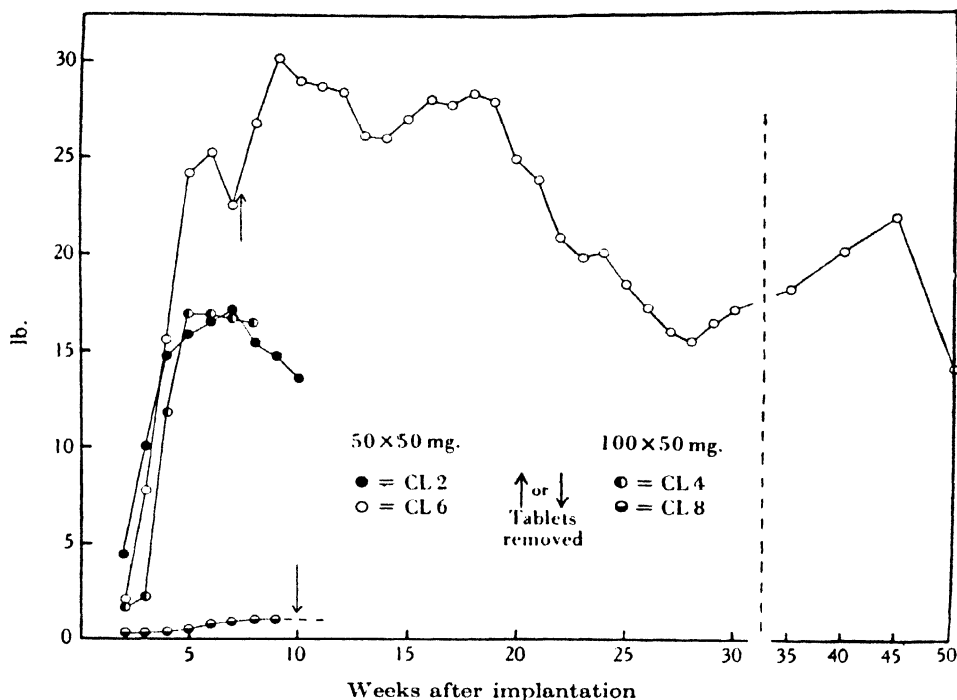


Fig. 20. 16—Lactation curves for dry cows in which lactation had been initiated by subcutaneous implantation of the stated number of tablets of diethylstilboestrol. Mean daily yields over 7-day periods are plotted. CL6 gave 7,400 lb. of milk in 365 days. (From Folley and Malpress, 1944a.)

Combined treatment with oestrogen and crude ox anterior-pituitary extract induces lactation more quickly than treatment with oestrogen alone (Folley *et al.* 1945a). There is evidence that some animals which fail to respond to oestrogen may be then brought into secretion by anterior-pituitary treatment (Folley and Young, 1941b; Folley *et al.* 1945a); moreover, animals in artificially induced lactation respond to the galactopoietic action of anterior-pituitary extracts (Lewis and Turner, 1942a) even at the peak of lactation (Folley and Young, 1941b; Folley *et al.* 1945a). Subsequent administration of oestrogen to animals previously brought artificially into lactation has in some cases resulted in further increases in milk yield (Walker and Stanley, 1941).

As regards the mechanism of the interesting effects of oestrogen discussed in this section, it is virtually certain that they are mediated by changes in the secretion by the anterior pituitary of hormones concerned in the initiation and maintenance of lactation, though it remains possible that the inhibitory effect of high doses of oestrogen may be partly due, as Nelson (1936) contended, to a direct action on the mammary gland. It seems likely, as suggested by Folley (1941), that the threshold for pituitary inhibition is higher than for stimulation, so that the nature of the effect of oestrogen treatment on mammary function would largely depend on the level of oestrogen attained in the body fluids and the duration of treatment. In general, high levels are likely to produce inhibition and lower levels, stimulation. Various phenomena encountered in the course of studies on the artificial induction of lactation, such as the rise in milk yield which often follows cessation of oestrogen treatment (Folley and Malpress, 1944a, b; Folley *et al.* 1945a; Day and Hammond, Jr., 1945), are in general accord with this concept, as are the results of Stanley and Owen (1941) and Mixner *et al.* (1944). It is not clear at present whether the synthetic oestrogens differ from the natural oestrogens, as Lewis and Turner (1940) affirm, in possessing the property of initiating lactation. The results of Frazier and Mu (1935), who used natural oestrogens, indicate that they do not.

Androgen

Testosterone inhibits lactation in mice (Robson, 1937) and rats (Folley and Kon, 1937; Edelmann and Gaunt, 1941; Huffman, 1941), whether the ovary is present or not (Edelmann and Gaunt, 1941). Androsterone in equivalent doses has little or no effect on lactation in either species (Robson, 1937; Folley and Kon, 1937). As we have seen previously, these two steroids show similar differences as regards mammogenic properties. This, together with their findings regarding the effects on lactation of oestrogen and progesterone respectively, led Folley and Kon (1937) to the generalisation that substances which promote mammary growth also inhibit lactation. When later it was found that deoxycorticosterone, pregnenolone and, at high dose levels, progesterone, stimulated mammary growth, an opportunity of testing this theory arose. Nelson (1941c) and Nelson *et al.* (1943) reported that, in accord with the theory, deoxycorticosterone would inhibit lactation in the guinea-pig. In the lactating rat, however, Folley (1942) found no evidence of inhibition with doses of progesterone of the order of those necessary for mammary growth, nor with 10 mg. deoxycorticosterone acetate daily, but pregnenolone exerted a slight inhibitory effect (*see also* Cohen and Stein, 1940). This generalisation would therefore appear to be untenable unless it proves that the thresholds for lactation inhibition are in general higher than those necessary for mammary growth.

Androgens have been used clinically for the inhibition of lactation in women (*see, for example*, Kurzrok and O'Connell, 1938).

Adrenal Cortex

Early studies of the physiology of the adrenal cortex showed that the physiological disturbances resulting from adrenalectomy include failure to lactate normally (Carr, 1931a; Swingle and Pfiffner, 1932; Brownell, Lockwood and Hartman, 1933; Gaunt, 1933; Britton and Kline, 1936). Later studies on the rat have

shown that the lactational failure resulting from adrenalectomy, though always serious, is not complete (Fig. 20, 17), though complete abolition may be approached in exceptional cases (Levenstein, 1937; Gaunt, 1941; Gaunt, Eversole and Kendall, 1942; Folley and Cowie, 1944; Cowie and Folley, 1947a). Effects of varying severity have been reported from different laboratories (compare Gaunt *et al.* 1942 with Folley and Cowie, 1944) but Cowie and Folley (1947a) have shown that they are not constant even in one rat colony. These workers observed variations in the intensity of lactation in their normal rats from time to time, and the degree of lactational inhibition due to adrenalectomy was inversely related to the lactational performance of simultaneously studied normal controls.

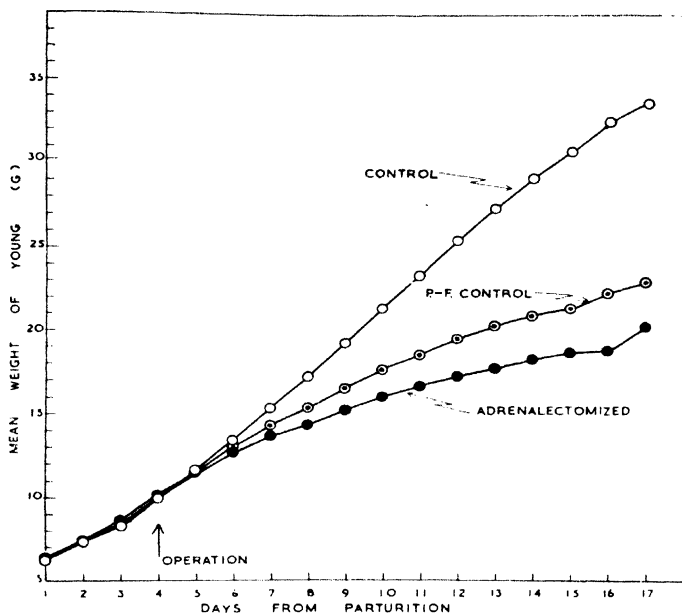


Fig. 20, 17--Effect of adrenalectomy on lactation as shown by the mean growth curves of litters of groups of sham-operated rats (upper curve), adrenalectomised rats (lower curve) and sham-operated rats pair-fed with the adrenalectomised rats (middle curve).

The first reports on the ability of cortical extracts to support lactation in adrenalectomised animals were conflicting. Life-maintaining extracts tested by Carr (1931b) and Brownell *et al.* (1933) were without effect, but successful replacement therapy was reported by Swingle and Pfiffner (1932) and Britton and Kline (1936). Gaunt and Tobin (1936) did much to clarify the position when they showed that considerably more extract was necessary to support lactation than is required for maintenance of life. At this time the best-known function of the adrenal cortex was the regulation of fluid and electrolyte exchanges, and Gaunt and Tobin (1936) pointed out that the known facts regarding the relation of the adrenal cortex to lactation could be interpreted on this basis since lactation involves considerable loss of fluids and electrolytes from the body. In support of this theory Gaunt and Tobin (1936) also showed that administration of salt

improves lactation in adrenalectomised rats (*see also* Levenstein, 1937; Folley and Cowie, 1944) and that the effectiveness of cortical extract in supporting lactation was increased if salt were given as well. In subsequent studies Gaunt *et al.* (1942) have confirmed the ability of cortical extracts to support normal lactation in adrenalectomised rats, but Folley and Cowie (1944) could only obtain partial success (confirmed by Cowie and Folley, 1947b).

The recent separation and characterisation of a number of crystalline steroids from adrenal cortex extracts has stimulated further analysis of the rôle of the adrenals in lactation. Gaunt (1941) reported that deoxycorticosterone caused no improvement in lactation in adrenalectomised rats, but later Gaunt *et al.* (1942) found partial restoration of lactation in a majority of their treated animals. The results were irregular, however, and bore no relation to the dose administered. On the other hand, Cowie and Folley (1947a) have shown that in their rats deoxycorticosterone will afford considerable, though usually incomplete, protection against the effects of adrenalectomy on lactation (*see also* Folley and Cowie, 1944). They demonstrated a linear relation between log. dose and response and found that the latter varies directly with the severity of the lactational failure due to adrenalectomy. In an experiment in which the effects of adrenalectomy were particularly marked, 3.0 mg. deoxycorticosterone acetate daily gave complete restoration of lactation (Cowie and Folley, 1947a). In contrast to their results with deoxycorticosterone, Gaunt *et al.* (1942) obtained complete restoration of lactation with 17-hydroxy-11-dehydrocorticosterone and they thus conclude that while the restoration of normal electrolyte metabolism is helpful for maintenance of normal lactation, the limiting factor for maximal secretion is a sufficiency of those cortical hormones, the 11-oxygenated steroids, which are concerned in carbohydrate metabolism, particularly in gluconeogenesis. Incidentally, the 11-oxygenated cortical steroids appear also to be more important than deoxycorticosterone as regards the initiation of lactation in the hypophysectomised guinea-pig (Nelson *et al.* 1943). Folley and Cowie (1944) have, however, found 11-oxygenated cortical steroids inferior to deoxycorticosterone for maintenance of lactation in their adrenalectomised rats, even when the latter receive a diet containing 50 per cent. protein, which should provide conditions particularly favourable for the action of agents which promote gluconeogenesis (Cowie and Folley, 1947b).

The existence of a specific lactation hormone of the adrenal cortex, cortilactin, has been postulated by Brownell *et al.* (1933) (*see also* Hartman *et al.* 1933). They claim that life-maintaining cortical extracts will only support lactation in the presence of a fraction which is removed by chilling at -12° C. Recently, Spoor *et al.* (1941) reported that cortilactin is a pigeon crop-stimulating hormone which can be separated from cortical extracts by isoelectric precipitation at pH 5.5. They claim that this substance, which does not promote gluconeogenesis and which they consider to be different from prolactin, supports lactation in adrenalectomised rats maintained with cortical extracts. However, since Hurst *et al.* (1942) failed to detect crop-stimulating activity in cortical extracts, and in view of claims to have effected complete restoration of lactation in adrenalectomised rats by administration of crystalline steroids (Gaunt *et al.* 1942; Cowie and Folley, 1947a), further evidence will be necessary before the existence of a specific lactation hormone of the adrenal cortex can be taken as established.

It can hardly be said that much progress has yet been made with the elucidation of the mechanism of the lactational failure resulting from adrenalectomy. It seems that failure of the pituitary to secrete prolactin is not involved since Gaunt and Tobin (1936) found that prolactin did not improve lactation in adrenalectomised rats maintained with cortical extract and since Meites *et al.* (1942) observed a *post-partum* rise in pituitary prolactin in adrenalectomised rats.

The replacement studies discussed above suggest that the crucial factor in some circumstances may be an upset in carbohydrate metabolism, in others a disturbance of some function more particularly related to the action of deoxy-corticosterone. Tissue enzyme studies by Folley and Greenbaum (1946) on adrenalectomised lactating rats receiving treatment with various adrenal steroids provide some indication that the adrenal cortex may affect lactation directly through the mammary gland arginase and indirectly through the liver arginase.

Thyroid

Evidence as to the effect of thyroidectomy on lactation is somewhat conflicting. As regards earlier work, observations (hardly critical, however, regarding the point at issue) which indicate that thyroidectomised animals lactate more or less normally have been made on the mouse (Davenport and Swingle, 1927), rabbit (Richon and Jeandelize, 1904), and dog (Dragstedt *et al.* 1924), while marked interference with lactation was noted in the rat (Hammett, 1922; Rickey, 1925) and goat (Zietzschmann, 1907; Grimmer, 1918; Trautmann, 1919; von Fellenberg and Grüter, 1932). Recent studies have scarcely clarified the situation. In the rat, Nelson and Tobin (1937) observed normal lactation after thyroidectomy (confirmed by Nelson, 1939b), while serious depression but not complete abolition of lactation was reported by Folley (1938), Folley, Scott Watson and Amoroso (1942) and Karnofsky (1942). Preheim (1940) reported lactational disturbances which were relatively slight. Temporary and relatively slight effects on milk yield following thyroidectomy in the goat were observed by Ralston, Cowser, Ragsdale, Herman and Turner (1940). In the cow, Graham Jnr. (1934a) reported a decrease in milk production following thyroidectomy, but it is significant that the effects of a control operation were almost as great. On the other hand, the experiments of Spielman *et al.* (1944) indicate that thyroidectomy lowers the milk yield and shortens the lactation period of the cow.

It is not clear how far the post-thyroidectomy effects on milk yield, which many have observed in farm animals, were due to operative disturbances. Marked temporary effects can almost certainly be ascribed to this cause. This possibility was certainly considered by Grimmer (1918) and the importance of operative disturbances is also evident from the report of Graham Jnr. (1934a). Spielman *et al.* (1944), however, obtained evidence of sub-normal lactation in thyroidectomised cows long after operative disturbances must have subsided. There is the further possibility that negative results might be due to incomplete thyroid removal or to the presence of accessory thyroid tissue; the histological studies of Folley *et al.* (1942) and Karnofsky (1942) indicate that complete thyroid removal is very difficult, if not impossible, in the rat. A further complication is the concomitant removal of the parathyroid tissue which itself may adversely affect lactation. This is particularly important in the rat in which there are only two parathyroids,

each embedded in a thyroid lobe, and it may be noted that Folley *et al.* (1942) found that the lactational deficiencies resulting from thyroidectomy in their rats could be partly alleviated by parathyroid extract.

On the whole, the available results indicate that lactation is possible in the absence of the thyroids but only at a somewhat reduced level. The thyroid hormone does not seem to be essential for the initiation of lactation since Houssay (1935b) induced milk secretion in thyroidectomised bitches by treatment with anterior-pituitary extract. Here there could be no question of foetal thyroids substituting for the maternal glands.

There is much more agreement about the galactopoietic effects of thyroid hormone administration. Many years ago it was claimed (Hertoghe, 1896) that the mild yield of the lactating cow could be increased by thyroid feeding. What was perhaps the first unequivocal demonstration of galactopoiesis resulting from thyroid feeding was given by Graham Jnr. (1934a) who observed considerable increases in milk production and even greater increases in milk fat production by cows fed dried thyroid gland. Injection of thyroxine gave similar results (Graham Jnr., 1934b). These results were extended by Folley and White (1936) who not only obtained striking confirmation of the increase in milk yield and fat percentage due to thyroxine injections but also showed that the non-fatty solids content of the milk increased during the treatment. Further extensive investigation of the galactopoietic effects in cows has left no doubt of their essential reproducibility under suitable conditions (Herman, Graham Jnr. and Turner, 1938; Hurst, Reece and Bartlett, 1940; Ralston *et al.* 1940; Smith and Dastur, 1940), while more recently, essentially similar results have been obtained in extensive studies involving the feeding of iodinated proteins (notably iodocasein) which exhibit biological activity characteristic of the thyroid hormone (Reineke and Turner, 1942a, b; Blaxter, 1943, 1945a, b, 1946; Reece, 1944; Van Landingham, Henderson and Weakley Jnr., 1944). The galactopoietic effects of anterior-pituitary thyrotrophin preparations noted by Folley and Young (1938, 1939) are, on the face of it, in accord with the above results but it is not certain that the responses were mediated by the thyroid since the relative galactopoietic activities of the thyrotrophic extracts bore no relation to their relative thyrotrophic potencies (measured by a method which, however, may not give a satisfactory indication of ability to promote the secretion of thyroid hormone [see Heyl and Laqueur, 1935]) and since the characteristic increase in milk fat content was not observed.

The galactopoietic effect of thyroid treatment in cows is most noticeable during the declining phase of lactation, the effect in early lactation being relatively insignificant (Graham Jnr., 1934a; Herman *et al.* 1938; Ralston *et al.* 1940). The response decreases absolutely, but increases relatively to the initial milk yield as lactation advances and the milk yield falls (Blaxter, 1945b). Thyroxine treatment does not prevent the normal decline in lactation but temporarily shifts the declining lactation curve upwards (Folley and White, 1936). The fat metabolism of the mammary gland is more sensitive to stimulation by thyroid hormone than the mechanisms for the synthesis of protein and carbohydrate since the increase in the milk non-fatty solids content is relatively small (but nevertheless unmistakable when investigated by proper methods [Folley and White, 1936; Blaxter, 1945a]) while the fat percentage may, under favourable conditions, increase to such an extent that the total daily fat production increases by as much as 50 per

cent. (Folley and White, 1936). Overdosage with thyroxine causes a decrease in milk yield (Graham Jnr., 1934a; de Fremery, 1936) which may explain clinical reports of lactational inhibition following thyroid administration.

The mechanism of the galactopoietic effect of the thyroid hormone is still a matter of conjecture. Various possibilities may be considered. In the first place it is reasonable to suppose that an increase in the metabolic rate of the mammary alveolar cells would result in an increased rate of milk synthesis, so that the galactopoietic action of the thyroid hormone would represent a specialised aspect of its well-known function as a stimulant of body metabolism. This might well apply particularly to the effects on milk fat, the secretion of which, as we have seen, is particularly susceptible to stimulation by thyroid treatment, since there is some evidence (for citations *see* Graham Jnr., 1934a) that, in the cow, milk fat production increases in circumstances in which an increase in B.M.R. might be expected and vice versa. Another possibility is that thyroid treatment may increase the supply of milk precursors to the mammary gland. This could be brought about by an increase in the rate of blood flow through the gland and also by virtue of an elevation of the level of such precursors in the blood. A considerable acceleration of heart rate accompanying the galactopoietic response to thyroid treatment was observed by Folley and White (1936) and most subsequent workers, which would imply an increase in the mammary circulation rate, and it is noteworthy that Fuller (1928) observed a positive correlation between pulse rate and milk yield in the cow. If the supply of milk precursors is a limiting factor in the declining phase of lactation it seems possible that an increased supply resulting from one or both of the above-mentioned causes might enhance the rate of milk secretion. Equally, if an increase in the metabolic activities of the milk-producing tissue led to an increase in the rate of milk synthesis an increased supply of milk precursors would be needed. Finally, there is the probability that galactopoiesis might result from interaction of the thyroid hormone with the endocrine system, probably by way of the anterior pituitary. For example, it is conceivable that a moderate degree of hyper-metabolism resulting from thyroid treatment might be accompanied by increased secretion of the galactopoietic complex by the anterior pituitary. What is virtually certain is that the galactopoietic effect under discussion is the result of a very complex readjustment of intermediary metabolic processes in which alterations in the activity of more than one endocrine gland are involved.

Parathyroids

Lactation involves a heavy drain of calcium and phosphorus from the body and it is to be expected that the integrity of the parathyroids would be essential for normal lactation. However, the relation of the parathyroids to lactation has been very little studied.

Such investigations as those of Dragstedt *et al.* (1924) and Kozelka, Hart and Bohstedt (1933) showed that thyroparathyroidectomised bitches would rear puppies if tetany were prevented by suitable methods (administration of calcium lactate or vitamin D), but since the situation was complicated by loss of the thyroids the results tell us little about the relationship between the parathyroids and lactation except, perhaps, that some milk can be secreted in the absence of the parathyroid hormone.

A little more has recently been learnt from the rat. The experiments of Folley *et al.* (1942) strongly suggested that the impairment of lactation observed by them and other workers in thyroparathyroidectomised rats might be due to loss of the parathyroids, since there was an indication that the lactational failure was less severe in the minority of rats possessing accessory parathyroid remnants, but more particularly because lactation could be partially restored by parathyroid hormone. These findings added significance to the earlier observations of Chandler (1932) that the young of parathyroidectomised rats were often abnormally small. Direct and conclusive evidence of the effect on lactation of parathyroid removal has recently been obtained by Cowie and Folley (1945) whose quantitative studies show that lactation is severely impaired but not completely abolished by parathyroidectomy. This would suggest that those workers who found lactation to be unaffected by thyroparathyroidectomy may have used a strain of rat in which accessory parathyroids were the rule rather than the exception.

Such evidence as is available then suggests that the parathyroid hormone has no direct action on the mammary gland but is concerned with lactation only indirectly and in so far as optimal secretion requires the maintenance of a normal calcium and phosphorus metabolism. In this connection it is a curious fact that Dragstedt's (1927) contention that lactation is a particularly effective stimulus for the conversion of a latent tetany into an acute condition does not seem to apply to the rat. Tetany is rarely observed during lactation in thyroparathyroidectomised or parathyroidectomised rats (Nelson and Tobin, 1937; Folley *et al.*, 1942; Cowie and Folley, 1945) except when parathyroid hormone has been given for a time and then withheld (Folley *et al.* 1942). The parathyroidectomised bitch seemingly reacts to the crisis of lactation by going into tetany, the rat by refusal to lactate.

The Endocrine Pancreas

Present knowledge indicates that insulin may influence the mammary gland in two ways; indirectly by virtue of its effect on general intermediary metabolism, by which the supply of milk precursors may be governed, and directly through its rôle in the carbohydrate metabolism of the mammary gland itself. Thus large doses of insulin decrease the milk yield of the cow (Gowen and Tobey, 1931b; Brown, Petersen and Gortner, 1936a), presumably mainly because the resulting hypoglycaemia is incompatible with the satisfaction of the considerable glucose requirements of the lactating udder. Evidence (*see* page 612) indicating a *direct* participation of insulin in the synthesis of lactose in the mammary gland has recently been reported by Knodt and Petersen (1946b).

Observations incidental to studies of insulin requirements of depancreatized bitches during pregnancy and lactation indicate that lactation may be initiated and puppies in many cases reared, provided sufficient insulin is given (Markowitz and Simpson, 1925; Cuthbert, Ivy, Isaacs and Gray, 1936; but see contrary observations by Markowitz and Soskin, 1927). Two investigations on the hormonal initiation of lactation in depancreatized bitches receiving insulin have given contradictory results. Chaikoff and Lyons (1933) failed in five cases out of six to initiate lactation with larger doses of prolactin than were used successfully in intact bitches. Rejecting the remote possibility that the pancreas might secrete a hormone, other than insulin, directly affecting the mammary glands,

these authors suggested that their negative results might be due to metabolic disturbances occurring despite the administration of insulin. But it seems possible that these failures might have been due to lack of mammary development since Nelson, Himwich and Fazekas (1936) were regularly able to initiate lactation with prolactin in depancreatized bitches receiving insulin provided that they were pseudo-pregnant and thus possessed well-developed mammary glands. Bitches in other phases of the oestrous cycle possessed poorly-developed mammary glands and failed to lactate in response to prolactin. Folley and Greenbaum (unpublished work) have initiated lactation by oestrogen treatment in a virgin goat rendered diabetic with alloxan and maintained with insulin.

Posterior Pituitary

Ott and Scott (1911) were the first to show that injection of posterior-pituitary extracts produces a rapid but evanescent action on the lactating mammary gland. They found that a single intravenous injection into a lactating goat with a cannulated teat, gave an almost immediate increase in the flow of milk through the cannula. The effect was transitory, however, lasting in their experiments for a few seconds only, and a repeat dose gave a diminished effect. This discovery was quickly confirmed in the cat by Schäfer and Mackenzie (1911) and Mackenzie (1911). Subsequent experiments on the goat (Hammond, 1913; Hill and Simpson, 1914; Maxwell and Rothera, 1915; Gaines, 1915) and cow (Turner and Slaughter, 1930) showed that posterior-pituitary extract injected into an animal which had been milked out would enable an additional quantity of milk to be obtained, or if injected into an animal just prior to milking would increase the yield at that milking above the expected amount. Again, a feature of the results was the evanescent nature of the responses and there was some indication (Hammond, 1913; Hill and Simpson, 1914; Turner and Slaughter, 1930) that extra milk obtained in response to posterior-pituitary extract at or following a milking was compensated for by a decreased yield at the next milking. There was little agreement as to whether or not the compensation was exact but in any event experiments on cows by Gavin (1913) indicated that repeated injections of posterior-lobe extract gave no increase in yield over a long period, a conclusion supported by modern experiments (Shaw, 1942a).

The effect of posterior-pituitary extract on the lactating mammary gland was at first regarded as a true galactopoietic effect, i.e. it was tacitly assumed that the extract temporarily stimulated the secretory activities of the alveolar cells, but since such extracts were known to cause contraction of smooth muscle tissue the alternative possibility that the effect merely represented the squeezing of otherwise unavailable preformed milk from the alveoli and finest ducts by virtue of the contraction of muscle tissue present in the mammary gland, soon came under consideration. At this time the question as to whether muscle fibres existed in close association with the mammary alveolar elements was under dispute (*see* Turner, 1939a, for discussion), but work by Swanson and Turner (1941) would appear to indicate that myo-epithelial cells, which might be capable of contraction under the influence of posterior-lobe hormones, are indeed present. The second of these alternative theories of the mode of action of posterior-pituitary extracts on the mammary gland was considered by Simpson and Hill (1915), but rejected because they were unable to simulate the effects of posterior-pituitary

extract by injection of BaCl_2 , a muscle stimulant. Others to consider this hypothesis only to reject it for one reason or another were Maxwell and Rothera (1915) and Hammond (1913). The first clearly to enunciate the currently accepted view that posterior-lobe extract has no direct effect on the secretory activities of the alveolar cells but causes discharge of milk from the gland by virtue of its ability to evoke contraction of contractile tissue surrounding the alveoli, appears to have been Heaney (1913) who, by means of a special apparatus, demonstrated a decrease in breast volume in women following the injection of posterior-lobe extract. Schäfer (1915), who gave reasons for discounting the BaCl_2 experiment of Simpson and Hill (1915) also, apparently independently, advanced the same theory, mainly because a second injection of posterior-lobe extract soon after the emptying of the gland due to a previous treatment did not produce any more milk. Others to adopt the same view were Gaines (1915) and Turner and Slaughter (1930). Most of the well-established facts already discussed and others, such as the rise in the internal udder pressure following an injection of posterior-lobe extract (Gaines, 1915 ; Maxwell and Rothera, 1915), point to such an interpretation, and there is little doubt that it is correct. In keeping with this view are the experiments of Smith (1932) in the rat and Houssay (1935a) in the dog which indicate that milk secretion is possible in the absence of the posterior hypophysis.

Despite the fact that the mode of action of posterior-lobe extracts on the mammary gland has been understood in its essentials for thirty years, the theory that the posterior lobe may be concerned in the normal discharge of milk from the mammary gland during nursing or milking has only recently been enunciated, though Gaines (1915) in a remarkable paper came within an ace of doing so. Gaines demonstrated a striking parallelism between the effect on the mammary gland of treatment with posterior-pituitary extract and of suckling, in that both, after a short latent period, produced a rise in udder pressure associated with the possibility of sucklings obtaining milk ; i.e. both induced what is called the "let-down" of milk. He showed that suckling failed to stimulate the "let-down" in anaesthetised animals but that following an injection of posterior-lobe extract the "let-down" occurred and sucklings were able to obtain milk. But though he produced strong evidence that posterior-pituitary hormones may be concerned in the "let-down" of milk under normal conditions, Gaines omitted specifically to postulate this.

Ely and Petersen (1941) in experiments on cows showed that fright or injection of adrenalin just before milking resulted in a reduced yield due to failure of the subjects to "let-down" their milk. The "let-down" could, however, be subsequently induced by injection of posterior-lobe extract and the udder completely emptied. Preparations of the oxytocic hormone seemed to be more effective than extracts rich in pressor hormone. On the basis of these results Ely and Petersen advanced the theory that the ejection of milk from the mammary gland may be governed by a balance between circulating adrenalin and oxytocin, the secretion of the latter at milking time being due to a nervous reflex normally actuated by tactile stimulation of sensory nerve endings in the teat, but capable of being conditioned to respond to such stimuli as washing the udder or other events regularly associated with milking time. Further evidence that the normal "let-down" of milk is due to the secretion of oxytocin into the blood was provided by Petersen and Ludwick (1942) who, in udder perfusion experiments, showed

that addition of blood from a cow which had been stimulated in the normal way to "let-down" its milk caused an immediate flow of milk from the perfused udder. Blood from cows not so stimulated had no effect. In other perfusion experiments Petersen (1942) tested a number of drugs and found that acetylcholine in addition to oxytocin caused ejection of milk. Turner and Cooper (1941) have attempted to devise a method for assaying posterior-pituitary extracts for their power of causing contraction in the tissues of the mammary gland of the rabbit. Among three types of extract the relationship between "mammary gland contracting" and oxytocic potencies was not very close and the authors suggested the possibility that the mammary gland effects might be due to the action of a third factor present in both oxytocic and pressor preparations.

What part the particular function of the posterior pituitary under discussion may play indirectly in the normal secretory activities of the mammary gland is at present an intriguing question. Miller and Petersen (1941) found that the "let-down" in cows was incomplete if there was undue delay between the application of the stimulus for the "let-down" and the beginning of milking, possibly because of dissipation of the circulating oxytocin. It thus seems probable that repeated failure to remove all the available milk, either because of faulty technique on the part of the milker or innate deficiency at some point of the neuro-hormonal discharge mechanism, might lead to an accelerated involution of the mammary gland and a shortened period of lactation. If this be the explanation of some cases of lack of persistence of lactation in cows and perhaps partial failure of lactation in women it is still not certain whether the neural or hormonal component of the mechanism is the more likely to be at fault. It should be noted, however, that Knodt and Petersen (1944) were able to decrease for a time the rate of decline of lactation in cows showing unexpected lack of persistence by regularly emptying the udder after each milking by means of oxytocin. Other experiments indicating the possible indirect importance of the posterior pituitary in the maintenance of normal lactation are those of Gomez (1939, 1940) who found that hypophysectomised lactating rats receiving replacement therapy were able to maintain their litters longer if the treatment was supplemented with regular posterior-pituitary injections.

The Initiation of Lactation

Though secretory changes in the mammary parenchyma may begin about mid-way through pregnancy, copious lactation as ordinarily understood does not normally set in until shortly after parturition. The mechanism responsible for the onset of lactation and its timing has long been the subject of speculation.

Prior to the discovery of the lactogenic function of the anterior pituitary it was customary to regard the initiation of copious lactation as an event of passive nature which followed the removal at parturition of stimuli causing the gestational growth of the mammary glands or, more particularly, of some inhibitory influence only operative during pregnancy. The idea that the onset of lactation was due to the abolition of an inhibitory influence appears to have been first formulated by Hildebrandt (1904) who suggested that the products of conception exert an influence whereby the mammary gland cells are protected from autolytic disintegrative processes supposed to occur during active secretion. Halban (1905)

held that lactation develops after the removal of an inhibitor of placental origin, a theory which in its essentials is not in conflict with some more modern views.

Later, the corpus luteum was suggested as the source of an inhibitor and the work of Hammond (1917) and Drummond-Robinson and Asdell (1926) appeared to support this idea. The latter workers showed that, in the pregnant goat, ablation of the corpora lutea sufficiently late in pregnancy for the mammary alveolar system to have developed, resulted in lactation. Anselmino and Hoffmann (1936) also suggested, on hardly adequate grounds as we have seen (page 576), that the corpus luteum is responsible for the inhibition of lactation during pregnancy but were forced to conclude that progesterone could not be the responsible agent.

A new possibility arose when evidence was presented that oestrogens possess the ability to inhibit lactation (*see* page 576) and attention naturally became focused on oestrogen as the agent responsible for the suppression of lactation during pregnancy, particularly since studies of the excretion of oestrogen in the urine of pregnancy indicated a high production of oestrogen during the later stages of this condition. The discovery of the positive lactogenic stimulus emanating from the anterior pituitary, however, introduced a new factor into the situation of which it was necessary to take account in any theory seeking to explain the mechanism of the initiation of lactation.

The first theory in which an acceptable rôle was assigned to the hypophysis was that of Nelson who in 1936 gave a good account of a series of his earlier papers covering the development of his hypothesis (Nelson, 1936). Moore and Price (1932) had attempted to solve what is in many ways an analogous problem, namely, the mechanism controlling the oestrous cycle, by postulating a reciprocal relationship between hypophysis and ovary. This concept was utilised by Nelson, according to whose theory oestrogens, probably of placental origin, suppress lactation during pregnancy (*a*) by inhibiting the secretion of prolactin by the hypophysis and (*b*) by an inhibitory action exerted directly on the mammary gland. The removal of this inhibition consequent upon the decrease in the circulating oestrogen at parturition, for which there is ample evidence, permits the release of prolactin by the anterior hypophysis and thus the initiation of lactation. Nelson's (1936) review and his earlier papers cited therein should be consulted for the details of the considerable amount of experimental evidence he amassed in support of this theory and of concordant facts established by other workers. It only remains to say that the theory was successful in harmonising most of the facts known at that time.

It is clear that the main tenet by which Nelson's theory must stand or fall is the postulate that the production of prolactin by the anterior pituitary is suppressed by oestrogen. This contention has recently been called in question by Meites and Turner (1942c) mainly because Turner and his collaborators have regularly found (*see* page 571) that oestrogen, even in huge doses, far from decreasing, actually increases the prolactin potency of the pituitary. They also cast doubt on the reliability of much of the evidence suggesting that oestrogens inhibit lactation (*see* discussion on page 577) and in this connection cite certain species in which pregnancy and lactation commonly proceed together. In regard to this last point, it is well known that in cows which are both pregnant and lactating, there is a rapid decline in milk yield after the fifth month of pregnancy which

Gaines and Davidson (1926) attributed to the effect of a then unknown inhibiting hormone.

In a series of four papers Meites and Turner (1942c, d, e, f) advance a new theory of the initiation of lactation according to which oestrogen stimulates the secretion of prolactin by the anterior lobe but is prevented from doing so during pregnancy by the simultaneous presence of progesterone, which they find (Meites and Turner, 1942d) will antagonise the evocation by oestrogen of an increase in the prolactin potency of the pituitary if the progesterone/oestrone ratio is high enough. The theory holds that during pregnancy the prolactin output of the pituitary is thus insufficient to induce lactation, but at parturition the overriding luteal influence is removed leaving the circulating oestrogen free to stimulate prolactin secretion. It will be seen that this is essentially a modern version of the old theory which held that lactation is suppressed by the corpus luteum of pregnancy, and it may be noted that Selye (1940b) has also recently suggested that the initiation of lactation at parturition is a progesterone-withdrawal phenomenon. He observed not only mammary growth but also initiation of secretion in female rats receiving oestradiol, but in rats receiving progesterone as well, the mammae were better developed but secretory changes were entirely absent.

The interesting and ingenious theory of Meites and Turner is open to certain serious objections which hamper its unqualified acceptance. First, the theory clearly assigns the key rôle in the initiation of lactation to prolactin and overlooks the possibility that other anterior-pituitary hormones may be involved to an equal extent. It has been shown above (*see* page 572) that the status of prolactin as the sole, specific lactogenic hormone is, to say the least, questionable, and Turner himself in the face of his observation that the pituitary prolactin potency of the pregnant yet non-lactating mouse is higher than that of the virgin animal (Hurst and Turner, 1942) has admitted that other hormones must be involved in the initiation of lactation. Second, the validity of the theory depends on the reality of changes observed under various conditions in the prolactin potency of the pituitary in small animals and on the further assumption that these changes, if real, reflect actual alterations in prolactin secretion and are not merely storage effects. As regards the first point, Hall (1944b) has questioned whether the assay method used by Turner and his collaborators, involving the intradermal injection over the pigeon crop-gland of suspensions of pituitary tissue, is capable of giving even true relative values as between one gland and another, while the available evidence suggesting that the observed increases in pituitary prolactin content are accompanied by an increased secretion of prolactin into the blood (Meites and Turner, 1942a) is hardly convincing and needs further confirmation. Third, there is no evidence that at parturition the decrease in circulating progesterone precedes the decrease in oestrogen as the theory demands. Fourth, Meites and Turner (1942d) found that gravimetric progesterone/oestrone ratios of the order of 1000 : 1 were necessary for the former to suppress the effects of the latter on the prolactin potency of the pituitary. We have no idea what ratios actually obtain during pregnancy, but judging from the experimentally determined ratios for optimal mammary alveolar growth (*see* page 546), which is a typical pregnancy phenomenon, they may well be considerably lower than this—perhaps of the order of 40 : 1. Fifth, the theory takes no account of the possible rôle of the placenta

in the initiation of the secretory phase in late pregnancy, for which a certain amount of evidence exists (*see* page 552).

If the relevancy of apparent changes in the pituitary prolactin potency is regarded as needing further proof, the main facts regarding the initiation of copious lactation at parturition could be equally well explained by a modification of the original theory of Nelson incorporating the concept (Folley, 1941) that low levels of circulating oestrogen stimulate the secretion by the anterior pituitary of hormones concerned in lactogenesis and galactopoiesis, while high levels, on the other hand, inhibit such secretion. At about the time of parturition, the level of circulating oestrogen, previously above the threshold value for pituitary inhibition, will fall, and in passing through the range between the two thresholds will cause functional activation of the anterior pituitary with respect to the production of lactogenic hormones, thus initiating full lactation.

Yet another theory, at present in disfavour, pointed to the intervention of a uterine factor in the chain of events leading to normal lactation. Selye *et al.* (1934) showed that surgical removal of uterine contents from pregnant rats resulted in lactation but not if the uterus were immediately distended with paraffin. These authors urged that mechanical distension of the uterus must be considered as a factor causing suppression of lactation during gestation and pointed out that the distension of the uterine walls decreases just before parturition despite the continued growth of the foetuses. Neither Bradbury (1941) nor Greene (1941) was, however, able to confirm these observations. The results of Freud and Wijsenbeek (1938) also seem to exclude uterine distension as a factor inhibiting lactation during pregnancy. They transferred rat foetuses to the abdomen but noted that no lactation occurred until the foetuses were artificially delivered.

This discussion of the mechanism of the initiation of *post-partum* lactation may be rounded off by a brief consideration of the novel views of Petersen (1944) who emphasises the fact that the secretory phase begins well before parturition. He believes that the copious flow of milk following parturition is due to the beginning of the ejection of the alveolar contents under the influence of oxytocin secreted during labour. The milk flow thus initiated is believed to be maintained by the subsequent stimuli of nursing or milking which cause an outpouring of prolactin by the anterior pituitary (*see* page 593).

IV. NEURAL FACTORS IN LACTATION

There is no evidence of the existence of secretory nerves directly controlling the permeability or synthetic activities of the alveolar epithelium; conclusive evidence that mammary secretion is under humoral rather than neural control comes from experiments such as those of Ribbert (1898) and Stricker (1929), who showed that lactation can occur in transplanted mammae. But it should not be thought that the normal function of the mammary gland is entirely independent of the nervous system; the contrary is the case as we shall now see.

Sympathectomy and lactation.—Recently there has been a revival of interest in the possible rôle of the sympathetic nervous system in lactation. Basch (1906), who incidentally should be consulted for references to early papers on the innervation of the mammary gland, had found that extirpation of the coeliac sympathetic

ganglion had little effect on milk secretion except perhaps when performed soon after parturition. A more modern paper by Cannon and Bright (1931) reported a delayed impairment of lactation following sympathectomy in one cat and one bitch (the latter in two successive lactations). Actually these results tell us very little, since so few animals were involved and the lactational observations were hardly critical. Bacq (1932) reported that in the rat, removal of both abdominal and one of the thoracic sympathetic chains occasionally interfered with lactation; but this happened in only two cases and critical examination of his data leads to the conclusion that his results may well have been coincidental. The more recent results of Simeone and Ross (1938) were no more decisive. Gross impairment of lactation in the cat following various sympathectomy operations was the exception rather than the rule, and in only one animal of seven could involutionary changes in the mammary gland be demonstrated histologically after section of its sympathetic nerve supply. It would thus appear that there are no grounds whatever for concluding that sympathectomy interferes with lactation, since in none of the above-mentioned experiments were the experimental conditions such as to allow of any lactational phenomena being ascribed with certainty to the effects of the operation. This accords with the findings of Ely and Petersen (1941) who, in the cow, sectioned the centrifugal sympathetic fibres to one half of the udder and found that during the subsequent lactation the capacity of the denervated glands to secrete and eject milk was unimpaired.

The suckling stimulus and milk secretion.—We now proceed to consider the possibility that neural influences may be indirectly concerned in the control of lactation in so far as the secretion of hormones involved in the maintenance of milk secretion may be affected by nervous stimuli. Selye (1934) has shown that in lactating rats which are continuously suckled, but in which the main galactophores have been ligated, thus preventing the escape of milk, the characteristically rapid mammary involution and cessation of secretion, such as results when the young are removed from intact rats, does not occur. He also found that if some nipples were suckled, whether the corresponding galactophores had been ligated or not, secretion was maintained for some considerable time in the remaining glands where suckling had been prevented by excision of the nipples. Selye and his collaborators (for full discussion see Selye *et al.*, 1934) interpreted their results as indicating that the suckling stimulus excites a nervous reflex which elicits the secretion of prolactin by the anterior pituitary, thus maintaining the secretory activities of the mammary gland, and they suggest that the mammary involution which follows weaning of the young is due to removal of the suckling stimulus rather than to accumulation of milk in the gland. The effects of non-removal of milk had previously been emphasised by the work of Kuramitsu and Loeb (1921) in the guinea-pig and Hammond (Hammond and Marshall, 1925) in the rabbit, who observed rapid involution in glands, suckling of which had been prevented by covering the nipples with collodion, even though other glands in the same animal were suckled. Selye and McKeown (1934a) later pointed out that the effects of the accumulation of milk in ligated glands eventually cause mammary involution despite the continuation of suckling. It thus seems probable that in actual fact both causes contribute to the mammary regression which follows weaning of the young in experimental or domestic animals or the gradual self-weaning of the young under natural conditions. An additional and as yet unknown cause must be postulated

to account for the gradual decline in milk yield which is observed in farm animals despite the regular application of the milking stimulus and subsequent withdrawal of milk. Similarly, Selye and McKeown (1934a) found that in mice, though lactation may be prolonged for upwards of two months by the continual provision of actively suckling litters, mammary involution does eventually occur though histologically it is of an abnormal type.

Williams (1941) has confirmed in mice the effect of the nursing stimulus in retarding mammary involution, but his results appear to differ somewhat from those of Selye (1934) in that he found less diminution of secretory activity in suckled glands from which escape of milk was prevented than in unsuckled glands from the same animal. The effects of the suckling stimulus could be reproduced in essentials by irritation of the nipples by the application of turpentine (Hooker and Williams, 1940). Here again, when some only of the nipples were treated the effects were manifest to varying degrees in all mammary glands.

Certain other results are in harmony with the theory that suckling reflexly causes discharge of prolactin from the anterior pituitary. Administration of prolactin retards mammary involution and to some extent maintains secretion in mice from which the litters have been removed (Hooker and Williams, 1941; Williams, 1945). Further, on the assumption that changes in the pituitary prolactin potency in small animals, as at present determined, reflect changes in prolactin secretion, the observations of Meites and Turner (1942c, f) who found greater pituitary prolactin potencies in rats and rabbits suckling litters than in animals prevented from suckling, could also be cited in support of the theory. Also significant in this regard may be the finding of Selye and McKeown (1934b) that application of the suckling stimulus to adult, cyclic rats and mice caused mammary development, the oestrous cycles being interrupted by long dioestrous periods. This condition, which since it showed many features in common with pseudo-pregnancy resulting from sterile copulation was called "suckling pseudo-pregnancy," and which could only be induced in the presence of the ovary (Selye and McKeown, 1934a), is explicable on the basis of the lutetrophic action of prolactin (*see* page 567) secreted in response to the nursing stimulus.

Since there is considerable evidence that a complex of anterior-pituitary hormones, rather than prolactin alone, is concerned with the maintenance of lactation, it seems probable that if lactation is normally maintained by a reflex neuro-hormonal mechanism of the kind discussed, the reflex must call forth the discharge of other anterior-lobe hormones in addition to prolactin.

The discharge of milk from the mammary gland.—The involvement of nervous mechanisms in the discharge of milk from the mammary gland has been mentioned briefly in connection with the relation of the posterior pituitary to lactation (*see* page 587, *et seq.*). The phenomenon of the so-called "let-down" of milk has naturally been studied mostly in connection with the cow, but it is likely that similar considerations are generally applicable. The salient facts are briefly as follows. By cannulation of the teat, and in the absence of tactile stimulation of this or other teats in the same animal, only the milk present in the gland cistern and larger ducts can be drawn off; the full yield of milk consisting, in addition, of much of the milk present in the alveoli and finer ducts can only be obtained when the teat is subjected to tactile stimulation, i.e. when the milking or nursing stimulus is

applied. Manometer readings of the milk pressure show a steady rise between one milking and the next (Tgetgel, 1926), but shortly after the beginning of milking there is a sudden further rise in pressure (Gaines, 1915; Maxwell and Rothera 1915; Tgetgel, 1926) with which is associated the possibility of obtaining the full yield of milk. This phenomenon, which is known as the "let-down," may be identified with the active forcing of milk under pressure from the lumina of the alveoli and finer ducts against the forces of capillary attraction which would otherwise prevent its egress.

Largely because of failure to distinguish clearly between two distinct processes, the secretion of milk (which itself may be said to consist of two phases, the synthesis of milk and its passage from the cells into the alveolar lumen) and its discharge from the gland, it was once widely held that the rise in milk pressure in response to the milking stimulus was due to reflex secretion, during the actual milking process, of much of the milk then obtained. Hammond (1936) has collected much evidence, existing in the literature, which effectively disposes of this fallacy, the inherent improbability of which renders consideration of the refuting evidence unnecessary here. Those interested are referred to Hammond's article. Hammond (1936) further, after considering many lines of evidence for which his paper should be consulted, put forward the view that the "let-down" of milk is an active process due to a nervous reflex excited by stimulation of the teat. He envisaged a purely nervous arc which brought about the contraction of smooth muscle fibres running in conjunction with the venous system of the udder, thereby occluding the venous vessels and engorging the udder tissues with blood. This in turn was supposed to force the milk under pressure from the alveoli and fine ducts. It seems surprising that Hammond should have apparently overlooked the significance of the important paper by Gaines (1915) which described many interesting experiments on the mechanism of the discharge of milk, the most striking of which was the demonstration that though anaesthesia inhibited the "let-down" in a bitch, the puppies could obtain milk after the injection of posterior-pituitary extract. This work, together with the experiments of Heaney (1913) and Schäfer (1915) among others (pages 587 *et seq.*), indicated a mechanism for causing contraction of the mammary tissues for which, as suggested by Folley (1940), there was evidence ready to hand, thus obviating the need for postulating the existence of a complex mechanism involving the induction of a state of "erection" in the udder tissues for which the evidence can only be described as circumstantial and tenuous. It remained for Ely and Petersen (1941) to formulate what seems at the present time to be the most acceptable theory of the mechanism of milk discharge, a theory postulating a neuro-hormonal arc the centripetal portion of which is nervous and the centrifugal portion mainly hormonal. Stimulation of the teat (or, in the case of cows, other conditioned stimuli habitually associated with preparation for milking) is supposed to cause reflex secretion of oxytocin by the posterior pituitary which in turn is directly responsible for the "let-down."

The remarks of Hammond (1936) regarding the inhibition of his postulated reflex by impulses from the brain equally well apply to the neuro-hormonal mechanism under discussion. It is well known that the "let-down" in cows can be inhibited by factors such as fright, the approach of an unfamiliar milker, etc. Ely and Petersen (1941) believe that such stimuli may cause the reflex secretion of adrenalin which, they found, interferes with the "let-down."

There is thus evidence for the existence in connection with lactation of two neuro-hormonal mechanisms both actuated by stimulation of the teat. That responsible for the discharge of milk presents no difficulty from the anatomical point of view since the innervation of the posterior lobe with fibres passing from the hypothalamus through the pituitary stalk is well-established. The intervention of nervous impulses in the discharge of hormones by the anterior lobe is more problematical, but in this connection should be recalled the secretion of gonadotrophins responsible for ovulation in the rabbit in response to nervous stimuli arising during coitus.

It is clear that failure of the mechanism governing milk discharge will amount to failure of lactation even though secretory hormones are being secreted in adequate amounts by the anterior lobe. For this reason it is possible that the apparent failure of lactation observed by Herold (1939) and Desclin (1940) in rats after section of the hypophyseal stalk (incidentally not confirmed by Dempsey and Uotila, 1940) may have been due to failure of the discharge mechanism rather than to obliteration of the mechanism postulated by Selye *et al.* (1934), though against this interpretation may be cited the observations of Smith (1932) and Houssay (1935a) who found that lactation was possible after removal of the posterior lobe. Equally, this explanation might apply to the interesting experiments of Ingelbrecht (1935) who found that if in rats the spinal cord was resected at a level appropriate for the sensory denervation of the six posterior mammae, the young died, in spite of vigorous suckling, when the six anterior nipples were shielded. If, however, suckling was permitted at two of the thoracic glands milk could be obtained from the abdominal and inguinal glands also. It would appear from these experiments that the centripetal portion of the particular mechanism concerned, whichever of the two it may be, involves a spinal pathway.

V. THE LACTATION CURVE

Variations in milk yield.—Variations in the rate of milk secretion throughout lactation have naturally been most closely studied in farm animals; it is probable that similar phenomena could be observed in other mammalian species. In accordance with histological studies in various species indicating that mammary secretion begins before parturition, appreciable amounts of secretion can often be withdrawn from the udders of heifers during pregnancy and the yield appears to increase with regular milking (Woodman and Hammond, 1923; Asdell, 1925). Indeed, small amounts of a "serous" secretion which Woodman and Hammond (1922) have shown to be relatively rich in globulin and albumin (particularly the former) and to contain small quantities of casein, lactose and fat can often be obtained from the udders of virgin heifers. In this connection it may be noted that the occurrence of lactation in the virgin goat is not at all uncommon judging from the cases recorded by Asdell (1925) and from the author's own experience (*see* Folley *et al.* 1941b).

Woodman and Hammond (1923) and Asdell (1925) studied the composition of the *ante-partum* secretions obtained from the udders of heifers pregnant for the first time. In early pregnancy the secretion, which is usually only obtainable in small amounts, is a "serous" liquid similar in composition to that obtainable from the udders of maiden heifers. At about the twentieth week of pregnancy it becomes

a viscous honey-like secretion containing large amounts of globulin. If this secretion is withdrawn at intervals from the udder the amount increases towards the end of pregnancy, particularly in the last week, and approaches normal milk in composition (Asdell, 1925). The composition of *ante-partum* mammary secretions in the human has been studied by Widdows, Lowenfeld, Bond, Shiskin and Taylor (1935).

Woodman and Hammond and also Asdell concluded that colostrum is a mixture of the honey-like secretion with normal milk. The characteristic colostrum composition (*see below*) of the mammary secretion normally obtained just after parturition appears to be due to the fact that the *ante-partum* mammary

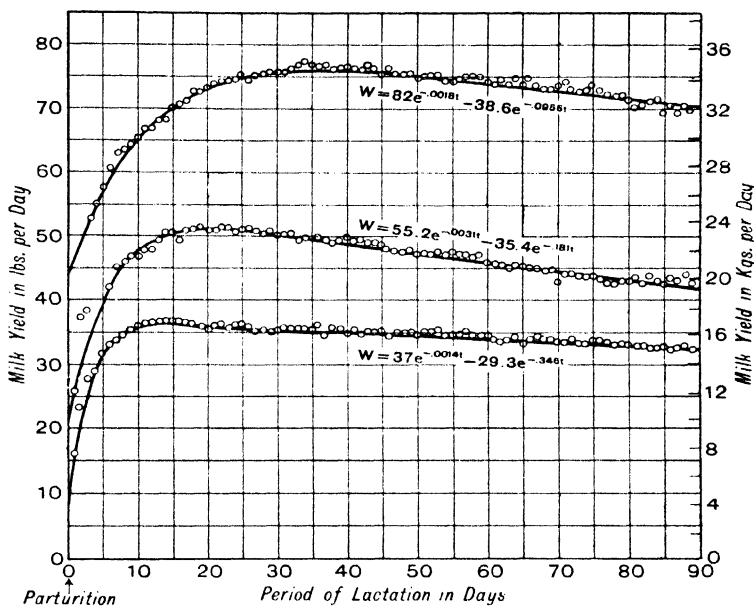


Fig. 20. 18— Mean lactation curves for groups of Holstein-Friesian cows. (After Brody, Turner and Ragsdale, 1924.)

secretions are not withdrawn from the gland. When, as in the experiments quoted above, secretion is regularly withdrawn from the mammary gland during pregnancy the fluid obtained at parturition is, to all intents and purposes, normal milk (Asdell, 1925). Petersen (1944) states that colostrum can be produced during lactation by suspending milking, the retained fluid tending to come into equilibrium with the blood (*see also* Petersen and Rigor, 1932).

Parturition is immediately followed by a striking increase in mammary secretion, the rate of which (and hence the daily milk yield) rises to a maximum, usually at two to four weeks *post-partum*. Thereafter a slow decline in milk yield sets in, which under constant conditions continues uninterruptedly over the remainder of the lactation period. Brody, Turner and Ragsdale (1924) studying the milk yields of groups of cows over the whole lactation period showed that the normal lactation curve can be fitted by the equation $M = Ae^{-k_1t} - Be^{-k_2t}$, where M is the milk yield at time t and A , B , k_1 and k_2 are constants (Fig. 20. 18).

Pregnancy has the effect of accelerating the normal decline in lactation in the cow, the effect according to Hammond and Sanders (1923) becoming appreciable at about the twentieth week from conception. The lactation yield of a pregnant cow is therefore less than it would be under similar conditions if she were allowed to remain barren. It seems more likely that the depressing effect of pregnancy on lactation is due to inhibitory hormones, as suggested by Gaines and Davidson (1926), than to the competition of the rapidly growing foetus for the nutrients of the maternal blood as Brody, Ragsdale and Turner (1923) were inclined to believe.

The lactation milk yield in the cow varies with age. Bartlett (1934b), analysing the results obtained over a large number of lactations, showed that the lactation yield on the average increases with each lactation to the seventh and thereafter declines rather sharply.

Variations in milk solids.—Bartlett (1929) has derived the normal lactation curve for milk fat percentage using data relating to large numbers of Dairy Shorthorn and Guernsey cows. The milk fat percentage falls slightly after parturition, reaching a minimum at a point roughly corresponding to the point of maximum milk yield, and then slowly increases during the period of declining lactation. It should be noted that Bartlett's figures related to cows that in most cases became pregnant during the lactation period. With successive lactations the mean lactation fat percentage appears slowly to decrease (Bartlett, 1934b).

The normal lactation curve for the non-fatty solids content of cows' milk as established by Bartlett (1934a) shows the following features. After parturition there is a slow fall in non-fatty solids content until about the eighth week *post-partum*. The mean values thereafter remain practically constant until the end of the lactation period is approached when in the case of pregnant cows there is a slight rise. The values for barren cows, on the other hand, show a further slight fall at the end of lactation. The mean lactation values for non-fatty solids content slowly decrease with increasing age of the cow (Bartlett, 1934b).

VI. THE NATURE AND CHEMICAL COMPOSITION OF MILK

Milk is an opaque white fluid which essentially consists of an emulsion of fat globules dispersed in an aqueous phase containing protein in colloidal solution and crystalloids in true solution. Milk contains three major constituents which are peculiar to it, namely, casein, lactose and milk fat. The first two substances are not found elsewhere in nature, while the particular mixtures of glycerides which constitute the milk fats of various species appear to be characteristic of milk. In addition to the major milk constituents, numerous other substances occur in milk in relatively small amounts.

For convenience the solids of milk can be considered as belonging to two classes, the fatty and non-fatty solids. Bovine milk fat is a complex mixture of glycerides, the fatty acids occurring there in largest amounts being oleic, myristic, palmitic and stearic acids, with smaller amounts of many others including butyric, capric, lauric and linoleic acids. Appreciable proportions of short-chain fatty acids (butyric and hexanoic) occur not only in bovine milk fat but also in the milk fat of other ruminants (Hilditch and Jasperson, 1944) but apparently not in that of non-ruminants such as man (Hilditch and Meara, 1944) and the horse (Hilditch and

Jaspersen, 1944). With the fatty solids of milk should also be included the small amounts of the fat-soluble vitamins and carotenoid pigments which normally occur in milk. The non-fatty solids consist of the phosphoprotein casein, the disaccharide lactose (4-galactosido-glucose), the proteins lactalbumin and lactoglobulin, various non-protein nitrogenous compounds, water-soluble vitamins and pigments, enzymes, and mineral salts such as calcium phosphate and sodium chloride. For further information on the chemistry of milk the reader is referred to Rogers (Associates of) (1935) and Davies (1939).

The average composition of milks of various species shows considerable variations which no doubt depend to a large extent on the varying needs of the young. Thus the milk of carnivores is in general characterised by high protein and low sugar content, while the milk of aquatic mammals such as the whale and porpoise contains much fat which is of high calorific value. The average composition of the milk of various species is given in Table I.

Colostrum.—Colostrum is the name given to the fluid secreted by the mammary gland during a short period following parturition when, as is normally the case, secretion has not been regularly removed from the gland during pregnancy. In composition, colostrum differs in important respects from milk, perhaps most strikingly in its high content of nitrogenous substances, chiefly consisting of globulin (serologically identical with blood serum globulin according to Crowther and Raistrick, 1916), and its low lactose content. Engel and Schlag (1925) studying changes in the composition of the fluid secreted by the bovine mammary gland during the colostrum period, i.e. the period of transition from colostrum to milk, which in the cow lasts about 6-12 days, found that the specific gravity and the concentration of total solids, protein, chlorides and ash, the values for which at parturition are considerably higher than those characteristic of normal milk, gradually fall, while the lactose content which at parturition is low, gradually rises.

The presence of formed bodies in colostrum was first described over a century ago. These bodies, which are sometimes globular in form and sometimes of irregular shape and which often contain granules and even nuclei, are known as colostrum corpuscles. During the period of transition from colostrum to milk, the corpuscles gradually disappear from the mammary secretion. Most of the earlier workers on the subject regarded colostrum corpuscles as transformed leucocytes and this view of their origin has been upheld by more recent workers such as Emmel, Weatherford and Streicher (1926). During lactation, when milk is regularly removed from the mammary gland, leucocytes passing into the alveolar tissue do not accumulate there but are withdrawn regularly in the milk. In the period prior to parturition, however, when the mammary gland is engorged with secretion which is not withdrawn, the leucocytes accumulate in the alveoli where, by ingestion of fat and other milk constituents, they become transformed into corpuscles; the same happens when nursing is suspended.

The physiological function of colostrum is of considerable interest. It was once believed that colostrum possessed laxative properties which were of value in enabling the newborn young to excrete faecal matter. Howe (1921), however, considered that though colostrum does not tend to delay defaecation in the newborn young as does the feeding of milk, it is not strictly a laxative. There is now considerable evidence that in many species ingestion of colostrum is of great

TABLE I

Percentage composition of milk of various species of mammals

(Adapted from Davies, 1939)

	Water.	Fat.	Sugar.	Casein.	Other protein.	Ash.
Man	88.50	3.30	6.80	0.90	0.40	0.20
Rabbit	—	16.71	1.98	8.17	2.21	—
Rat	68.3	14.8	2.8	9.2	2.6	1.5
Guinea-pig	—	7.31	2.31	4.60	0.49	—
Dog	75.44	9.57	3.09	6.10	5.05	0.73
Cat	81.63	3.33	4.91	3.12	5.96	0.58
Porpoise	41.11	48.50	1.33	11.19		0.57
Whale	48.67	43.67	—	7.11	—	0.46
Elephant	67.85	19.57	8.84	3.09		0.65
Ass	89.88	1.50	6.09	0.73	1.31	0.49
Mule	91.50	1.59	4.80	1.64		0.38
Horse	90.68	1.17	5.77	1.27	0.75	0.36
Zebra	—	4.80	5.34	3.03		—
Pig	84.04	4.55	3.13	7.23		1.05
Hippopotamus	—	4.51	—	—		—
Camel	86.57	3.07	5.59	4.00		0.77
Llama	86.55	3.15	5.60	3.00	0.90	0.80
Reindeer	68.20	17.10	2.08	8.40	2.00	1.50
Ox	87.32	3.75	4.75	3.00	0.40	0.75
Goat	82.34	7.57	4.96	3.62	0.60	0.84
Buffalo	86.04	4.63	4.22	3.49	0.86	0.76
Sheep	79.46	8.63	4.28	5.23	1.45	0.97

importance for the protection of the newborn animal against disease. In species such as the cow, horse and goat in which the placenta is relatively impermeable to antibodies, colostrum is of great importance as a means whereby maternal antibodies can be transmitted from the mother to her offspring during the short period after parturition when they can be absorbed from the alimentary tract

without undergoing destruction. In some other species, notably the human, colostrum seems to be of less importance in this respect since the transfer of immune bodies from mother to offspring appears to be mainly across the placenta. It is of interest to note that cows' colostrum contains very much more vitamin A and carotene than normal milk (Drummond, Coward and Watson, 1921 ; Dann, 1933), so that in the cow and possibly other species, ingestion of colostrum enables the young to build up a reserve of vitamin A soon after birth. Human colostrum, on the other hand, does not appear to differ very markedly from human milk as regards vitamin A content (Dann, 1936).

VII. THE MODE OF FORMATION OF MILK

Cytological Studies

As a result of cytological studies various theories have been advanced as to the mode of secretion of milk. For a detailed review of the historical aspect of this subject the reader is recommended to consult Turner (1939a) on whose account the following is, to some extent, based. It was at one time believed (e.g. Virchow, 1871) that milk was formed as a result of fatty degeneration of the alveolar epithelium. Heidenhain (1883) pointed out, however, that if this theory were true the alveolar epithelium would need very frequent renewal (perhaps five times daily) during milk secretion. Evidence of the active cell proliferation postulated by this theory has never been obtained ; Maeder (1922), for instance, emphasised the scarcity of mitotic and amitotic division in the epithelial cells of the lactating mammary gland of the rat. This theory of the origin of milk has long been abandoned and milk formation is now generally regarded as a process of true secretion.

Some workers have put forward theories which may be classed as "cell decapitation" theories. The essential element in theories of this type is the idea that during lactation portions of the alveolar epithelial cells come to project into the lumina ("cupola formation"). These "cupolas" become detached and fall with their contained secretory droplets into the alveolar lumina, the secretion being set free on degeneration of the detached fragment of cell cytoplasm. This type of theory was advanced by Paartsch (1880) and Heidenhain (1883) who described two types of cells which they observed in actively secreting epithelium, namely, tall columnar cells with well-defined cell walls and containing fat droplets, and flat cells with ill-defined cell walls. The latter cells were supposed to arise from the former by loss of cytoplasm containing secretory droplets. A somewhat similar theory, involving a three-stage secretory cycle, in which the height of the epithelial cells was thought to indicate their secretory activity was put forward by Limon (1902). More recent workers who have observed evidence of "cell decapitation" are Weatherford (1929) and Jeffers (1935a), while Beams' (1927) observation of the presence of Golgi material in the mammary alveoli during lactation would seem to support the view that some cellular cytoplasm is lost during secretion. Da Fano (1922) in an earlier study, however, could find no evidence of the loss of Golgi material with the secretion from the epithelial cells of the mammary gland.

Theories of milk secretion which postulated "cell decapitation" were, however, not acceptable to all workers. Benda (1894), Michaelis (1898), Arnold (1905) and Bertkau (1907) took the view that secretory droplets were normally extruded into the lumina from cells which remained intact. The latter considered that all evidences of loss of cellular cytoplasm during secretion were artifacts of histological fixation. A more recent study by Maeder (1922) of the mammary gland of the albino mouse led him to conclude that "cell decapitation" does not normally occur and that milk arises as a result of a purely secretory process of the alveolar



Fig. 20. 19.—Section of lactating alveolar epithelium (goat) showing tall epithelial cells at the beginning of the secretory cycle. Fat droplets are in process of release from the cytoplasm. $\times 1,760$.

epithelium. The observations of Weatherford (1929) on the rat seem to favour a combination of both types of theory, since they suggest that though loss of cytoplasm may occur during extrusion of secretion from the epithelial cells, the occurrence of this process is the exception rather than the rule.

Weatherford (1929) has carried out an extensive cytological study of the mammary gland of the albino rat in the several stages of development and activity. In the gland of the virgin animal the alveolar epithelium consists of low columnar or cuboidal cells, the ovoid nuclei being usually situated near the basal membranes. The Golgi apparatus consists of a granular network near the nucleus and often surrounding it. In primiparous animals during the first half of pregnancy, the alveoli increase in size and number, mitotic but not amitotic figures being frequent

in preparations from this stage. Weatherford observed very little epithelial cell proliferation during the second half of pregnancy, which agrees with the observations of Roberts (1921) who showed that though the alveoli continue to increase in size during late pregnancy, this is due to hypertrophy of the cells and increase in the size of the lumina owing to gradual accumulation of secretion. During pregnancy the epithelial cells lengthen and vacuoles of secretion begin to appear in the region of the Golgi apparatus, while at the same time the amount of Golgi material undergoes an increase. Just before parturition the epithelial cells are



Fig. 20. 20—Section of lactating alveolar epithelium (goat) at an intermediate stage of the secretory cycle. The alveolus is distended and the epithelial cells are becoming shorter and about to flatten. Fat is still being secreted. The capillary blood vessels are well shown beneath the epithelium. $\times 1,760$.

distended with secretion and the Golgi apparatus, which is now greatly hypertrophied, often appears to surround the vacuoles. Distortion of the cells with secretory droplets at this stage, often results in the nucleus being pushed towards the basement cell membrane.

During lactation there is a great increase in the size of the alveoli, and accumulation of secretion in the lumina may cause flattening of the epithelial cells. In preparations from lactating glands Weatherford could detect cells in which the membranes bordering the lumina showed irregular outlines presumably caused by rupture during discharge of secretion. At a later stage, the membranes are reconstituted and their contours become smooth. Three stages in the secretory cycle exhibited by mammary epithelial cells are illustrated in Figs. 20, 19, 20 and

21. Hypertrophy of the Golgi apparatus persists during lactation, Weatherford confirming Da Fano (1922) in this, and the results of the former indicate that secretory droplets arise within the meshes of the Golgi apparatus and move thence through the cell towards the lumen. As lactation declines, the proportion of low cuboidal cells in the epithelium increases. In the period of post-lactational involution the alveolar lumina become smaller and the Golgi material fragmented. Da Fano (1922) also noticed that involution was accompanied by the fragmentation of the Golgi material into small rounded bodies.



Fig. 20, 21.—Section of lactating alveolar epithelium (goat) showing alveolus at maximum distension. The epithelium is flattened and the surface cell membranes are more or less intact. $\times 1,760$.

The rôle of cell structures in lactation.—The fact that the nucleus is rich in phosphorus suggested to Nissen (1886) that it might play a part in the synthesis of casein. Observations of bi- and tri-nucleate cells in sections of lactating mammary tissue have been reported by Nissen and others, but other workers (e.g. Arnold, 1905) denied the frequent occurrence of bi-nucleate cells in mammary gland preparations. If the casein (and perhaps other constituents) of milk did arise from nuclear material as a result of nuclear degeneration, the occurrence of frequent mitosis during lactation would be expected, but as has been indicated above, most modern workers deny this.

Da Fano (1922) who studied the Golgi apparatus in the mammary epithelium of the rat, mouse and other mammals, observed hypertrophy of the Golgi material

during pregnancy and lactation and interpreted his observations as indicating that the Golgi apparatus was in some way involved in milk secretion. Later, Beams (1927) observed in the epithelial cells of lactating glands Golgi material surrounding fat droplets. The observations of Weatherford (1929) strongly suggest that the Golgi apparatus is concerned in the secretory process. Very noteworthy in this connection was the fact that the amount of Golgi material in the mammary epithelial cells of his rats varied with the intensity of their secretory activity, and that when the rate of secretion was experimentally retarded by suspension of nursing, the amount of Golgi material decreased. Judged by the amount of Golgi material in the alveolar epithelial cells, lactation in the rat appeared to reach its maximum rate at 8-10 days *post-partum* with a secondary rise at about the fourteenth day.

The presence of fuchsinophilic granules and filaments (mitochondria) was probably first observed in mammary gland cells by Altmann (1889). Later they were studied by Steinhaus (1892), Arnold (1905), Hoven (1911, 1912) and others. Weatherford (1929) included observations of mitochondria in his study of the mammary gland of the rat. His observations, on the whole, confirmed those of earlier workers in showing that during the resting state the mitochondria are chiefly granular and that during pregnancy and lactation there is a progressive appearance of filamentous mitochondria which are often orientated parallel to a proximo-distal axis of the cell. The filaments decrease in number as lactation declines. Some of the earlier workers believed that the mitochondria themselves became directly transformed into secretion, though Cowdry (1918) inclined to the view that the mitochondria play a rôle in the elaboration of cellular products more analogous to that of a catalytic surface. Weatherford, however, could not observe any of the intermediate stages of the transformation of mitochondria into secretory droplets, but his results do emphasise the close correlation between the secretory activity of the alveolar epithelium and the number and appearance of the mitochondria in the cells. Jeffers (1935a) observed chondriosomes in rat mammary tissue during pregnancy, lactation and involution.

Biochemical Studies

The mammary gland is an organ of particular interest from the point of view of intermediary metabolism, since, apart from vascular and nervous connections, it is a self-contained organ, exterior to the body, which during lactation is the site of very active synthesis of protein, fat and carbohydrate. Since the three major constituents of milk are peculiar to this fluid it is clear that they must be synthesised from precursors transported to the gland in the blood. Any of the reactions and transformations characteristic of anabolism or catabolism in the body as a whole must be considered as possibilities in connection with mammary gland metabolism. The further possibility that the mode of synthesis of a given milk constituent may vary under different conditions in a given species, and under comparable conditions between one species and another, should not be ignored.

Investigation of the biochemistry of milk synthesis has hardly progressed beyond the stage of attempted identification of the blood precursors of milk. Study of the intermediate stages of mammary gland metabolism is as yet in its infancy and an adequate attack upon this problem will scarcely be feasible until the nature of the milk precursors is known with greater certainty.

Most of the techniques used in metabolic studies of other organs and tissues have been applied to the mammary gland with varying degrees of success. The technique hitherto most widely used for mammary gland studies is based on the comparison of the concentration of suspected metabolites in the blood entering and leaving the gland. This, the so-called arterio-venous technique, has so far given most information and seems peculiarly suited to mammary gland studies since in farm animals the necessary blood samples can be taken without operative preparation. The perfusion technique has also been used to some extent (*see* Petersen, Shaw and Visscher, 1941, for a description of a modern apparatus) and Petersen and Shaw (1939) claim that it offers the advantage that general disturbance to the animal, which may affect arterio-venous (A-V) differences, is obviated. A certain amount of information has accrued from *in vitro* studies mostly involving attempted synthesis of milk constituents in the presence of mammary gland slices, pulp or extracts and also from studies on enzymes present in the latter. Experiments involving experimental alterations in the level of suspected milk precursors in the blood have given relatively little useful information. Tracer isotopes have so far been used in one or two studies only, but undoubtedly much progress in mammary gland biochemistry may be expected from the extended use of this technique in the future.

The arterio-venous technique as applied to the mammary gland.—Since this is easily the most important technique at present in use for the investigation of mammary gland metabolism, some consideration of it as applied to this purpose is necessary. The principle was first used in a study of mammary metabolism by Kaufmann and Magne (1906). Samples of mammary venous blood can readily be obtained in the cow and goat since one of the principal veins from the udder, the abdominal subcutaneous vein, is superficially placed on the abdomen; but the work of Kaufmann and Magne was carried out before the more difficult problem of obtaining arterial blood had been solved. Accordingly, these workers, wishing to determine the uptake of blood sugar by the udder of the lactating cow, compared the blood sugar content of mammary venous blood with that of blood from the jugular vein. On the assumption that the sugar requirements of the tissues of the head and neck for energy production were about equal to those of the lactating udder, they considered that the jugular blood sugar could be taken as representing that of arterial blood less the sugar used by the udder for the production of energy. They found that in the lactating cow, the mammary venous blood contained less sugar than the jugular blood and considered that the difference represented sugar used by the udder for purposes other than energy production. The original Kaufmann-Magne technique involving jugular-mammary venous comparisons has since been used by Meigs, Blatherwick and Cary (1919), Cary (1920), Nikitin (1935) and McCay and Maynard (1935).

Blackwood and Stirling (1932a) (*see also* Blackwood and Wishart, 1936) showed that the Kaufmann-Magne method may give misleading results because blood traversing the head and neck undergoes concentration due to loss of water in the saliva, so that the composition of jugular blood may, in important respects, be very different from that of arterial blood. As Lintzel (1934) has pointed out, evaporation from the naso-pharynx will doubtless contribute to this effect. Nevertheless, as recently as 1938, Maynard, McCay, Ellis, Hodson and Davis (1938) were of the opinion that the Kaufmann-Magne technique may be useful (presumably

for qualitative studies only) where large jugular-mammary venous differences are involved, since they consider that jugular blood samples can be taken with less disturbance to the cow than can arterial samples by the methods now to be enumerated.

Blackwood and Stirling (1932a, b) were the first to carry out true arterio-venous studies on the udder of the cow, the arterial blood being obtained from the radial artery. Other and more convenient methods of obtaining arterial blood for arterio-venous studies in cows have since been introduced by Graham Jnr., Kay and McIntosh (1936) who puncture the internal iliac artery through the rectal wall, Maynard *et al.* (1938) who puncture the internal pudic artery *per vaginam* and Shaw and Petersen (1938a, 1940) who puncture the pre-pudic artery *per rectum*. For arterio-venous studies in the goat Lintzel (1934) obtained arterial blood by cardiac puncture and Graham Jnr., Turner and Gomez (1937) from an exteriorised carotid artery.

Results obtained by application of the arterio-venous technique to the mammary gland must be evaluated in relation to certain important considerations that arise respecting its use. These are cited below as illustrating technical difficulties involved in the method and as exemplifying objections that may be raised against it on theoretical grounds, and they must be kept in mind in assessing the significance of the results obtained by its use.

First, the two blood samples must be taken as nearly simultaneously as possible because arterial blood composition appears to vary from time to time (Shaw and Petersen, 1939), the venous puncture being made first, so that the effect of any disturbances connected with the arterial sampling which might temporarily cause a cessation of milk secretion (*see* Graham Jnr., Kay and McIntosh, 1936) will be minimised, the venous sample having already been taken. Further, according to Graham Jnr., Kay and McIntosh (1936) the sampling should be completed with a minimum of delay otherwise blood volume changes occur, leading to untenable results.

Second, there is ample evidence that the blood samples must be taken with a minimum of disturbance to the subject if misleading results are to be avoided. Graham Jnr., Kay and McIntosh (1936) reported that disturbance in the course of bleeding causes milk secretion temporarily to cease with resulting diminution in and sometimes reversal of A-V differences for certain blood constituents. Shaw and Petersen (1939) found that, regardless of the rapidity with which the samples are taken, such disturbances are accompanied by fluid shifts (detected by changes in blood haemoglobin content) between the blood and udder tissues which particularly affect A-V differences in non-diffusible blood constituents such as fat and protein. Correction of the A-V differences for haemoconcentration or haemodilution produced results which were obviously untenable and it appears that results corrected for blood concentration changes, such as were reported by Graham Jnr., Peterson, Houchin and Turner (1938), may be of doubtful validity. In the absence of visible disturbance it has been generally found that the passage of water from blood to milk causes no appreciable concentration of the blood passing through the udder and it would seem that only experiments in which no detectable blood volume changes have occurred should be considered as valid (*see* Shaw and Petersen, 1939). Reineke, Williamson and Turner (1941a) who found that the rates of milk secretion and blood sugar utilisation in the goat were unaffected under nembutal anaesthesia

have proposed the use of the nembutalised goat for arterio-venous studies since blood samples can be taken under anaesthesia without disturbance.

Third, it may be objected that blood from the abdominal subcutaneous vein is not necessarily representative of the total venous blood of the mammary gland since this vein is not the sole venous outlet from the udder. Despite the fact that the existence of venous anastomoses in the udder tends to equalize the composition of the blood in the different veins this objection may be a serious one and it would be of interest to compare the composition of blood from the abdominal subcutaneous vein with that of the blood from an alternative exit such as the external pudic vein. An attempt at overcoming this difficulty was made by Graham Jnr. (1937) and Graham Jnr., Houchin and Turner (1937) who used unilaterally mastectomised goats in which all major veins from the remaining gland other than the abdominal subcutaneous vein were ligated. An even more serious objection, and one which it has not yet been possible to overcome, is the fact that a proportion of the blood constituents absorbed by the mammary gland from the blood must undoubtedly leave the gland in the lymph. Virtually nothing quantitative is known of the lymph drainage from the udder, but it is evident that the loss of metabolites in the lymph makes it impossible to conduct strictly accurate balance experiments on the mammary gland and will to an unknown extent reduce the significance of quantitative conclusions drawn from A-V differences.

Fourth, the apparent absorption of a blood constituent by the lactating udder, even if it can be shown that there is no uptake by the non-lactating gland, does not prove that the substance in question is a milk precursor. Quite apart from the possible passage of the substance into the lymph it may be used by the glandular tissues for purposes other than milk synthesis, such as energy production. Relatively little that is certain and quantitative is known about the energy requirements of the mammary gland. Shaw and Petersen (1938a, 1940) held that the gland obtains energy by the partial oxidation of fatty acids and, later, Shaw (1942b) obtained results consistent with the hypothesis that about 37 per cent. of the oxygen utilised by the lactating udder was used for the oxidation of β -hydroxybutyric acid (*see also* Shaw, Powell Jnr. and Knodt, 1942; Shaw and Petersen, 1943). In cows suffering from ketosis the udder is said to turn from the oxidation of higher fatty acids to the sole oxidation of β -hydroxybutyric acid (Shaw, 1942b), but this interpretation of the evidence may need revision in view of the fact that Knodt and Petersen (1946a) have reported the partial conversion of β -hydroxybutyric acid into acetone and acetoacetic acid (which are not utilised by the udder of the intact cow (Shaw, 1942b)) by mammary gland slices *in vitro*. Graham Jnr., Houchin, Peterson and Turner (1938) on the basis of the dubious assumption that the gland obtains its energy solely from the oxidation of carbohydrate, and using a formula that as printed appears to be in error, calculated that only about 10 per cent. of the energy taken from the blood is used in transforming blood constituents to milk constituents.

Fifth, in order to determine whether the rate of absorption of a suspected milk precursor by the udder is sufficient to account for the observed rate of synthesis of the corresponding milk constituent, the ratio of blood flow to milk secreted must be known. Average values for this ratio over a given period have been estimated for the goat by Graham Jnr., Peterson, Houchin and Turner (1938) from direct blood flow measurements as 150-250 : 1 (*see also* Graham Jnr., Houchin,

Peterson and Turner, 1938), values which are slightly higher than the ratios reported by Jung (1933). It should be noted that these measurements were made on the abdominal subcutaneous vein in goats operatively prepared as mentioned above and since there was some remaining peripheral drainage the results are probably somewhat low. Alternatively, the average blood/milk volume ratio can be indirectly calculated from A-V concentration differences for an element such as calcium which occurs both in blood and milk, provided the concentration of the element in the milk is also known. Ratios for the cow determined in this way from calcium uptake have been given as 387:1 (Shaw and Petersen, 1938a), 410:1 (Shaw and Petersen, 1940) and 488-650:1 (Shaw *et al.* 1942). Phosphorus uptake determinations have given ratios varying from 331-511:1 (Shaw *et al.* 1942). It seems likely that this method somewhat underestimates the true ratios since there is almost certain to be considerable passage of calcium and phosphorus into the lymph. Blood volume/milk volume ratios calculated from experimentally observed A-V differences for suspected milk precursors have been given by Lintzel (1934), Graham Jnr., Jones and Kay (1936) and Shaw and Petersen (1938a). Such calculations have no meaning except in so far as they are used for the purpose of comparison with an independent estimate of the blood/milk volume ratio as a test of a precursor hypothesis. To discuss them as independent estimates in their own right, as was done by the afore-mentioned authors as well as Petersen (1944) and Kay (1945), involves an obvious logical fallacy. Quite apart from difficulties associated with the determination of the blood/milk volume ratio, balance calculations of the type discussed above are subject to error due to the fact that single A-V determinations give approximately instantaneous values for the uptake of the blood constituent in question, while the milk data used in the calculations correspond to the milk secreted over a considerable period, during which the rate of milk secretion and precursor uptake (e.g. see Shaw and Petersen, 1940, for blood fat and calcium uptake) are certainly not constant.

In sum, the above considerations make it clear that there are considerable technical difficulties associated with the arterio-venous method as applied to the mammary gland and, further, that the results even of experiments which, in the light of present knowledge, are unobjectionable from the technical point of view are still open to a considerable degree of uncertainty as regards quantitative interpretation.

Carbohydrate metabolism of the mammary gland.—Bert (1884) amputated the udders of goats which he afterwards caused to become pregnant and bear young. For a short time immediately following parturition, he was able to detect a reducing substance in the urine, assumed by him to be glucose which in the intact lactating animal would be used for the synthesis of lactose. Similar observations were later made by Porcher (1909) with goats which had undergone mastectomy during pregnancy or lactation. Porcher claimed to have proved that the reducing substance appearing temporarily in the urine was glucose and not lactose and that the temporary glycosuria was accompanied by hyperglycaemia. Neither Bert, Porcher, nor Marshall and Kirkness (1907) were able to obtain similar results with the guinea-pig, and contradictory results with the sheep were reported by Foà (1912) and with the goat by Moore and Parker (1901). These discrepancies were discussed by Meigs (1922).

Kaufmann and Magne (1906) observed that the blood from the mammary vein in lactating cows contained less sugar than jugular blood, while in a dry cow the sugar contents of the two types of blood were nearly equal. These results were subsequently interpreted (Meigs, 1922) as evidence that blood sugar is the precursor of lactose.

Foà (1912) used the perfusion technique in an attempt to determine the precursor of lactose. He found that when a sheep's udder was perfused with a mixture of blood and Ringer's solution, milk containing lactose was secreted, while the sugar content of the perfusate decreased. Addition of dextrose to the perfusion fluid increased the lactose content of the secretion. When the gland was perfused with Ringer containing dextrose, an aqueous fluid containing lactose was secreted, but no lactose was secreted when the gland was perfused with Ringer containing galactose or with Ringer alone.

Modern work on the precursors of lactose dates from the arterio-venous studies of Blackwood and Stirling (1932b), who observed A-V differences in true blood sugar amounting to 5-15 mg./100 ml. in lactating cows and smaller differences in dry cows. In subsequent arterio-venous studies A-V blood sugar differences, on the whole of the same order, have been observed in lactating cows (Graham Jnr., Jones and Kay, 1936; Shaw, Boyd and Petersen, 1938; Maynard *et al.* 1938; Shaw *et al.* 1942) even in cases of hypoglycaemia associated with ketosis (Shaw, 1943), and in goats both normal (Lintzel, 1934; Graham Jnr., 1937) and under the influence of nembutal (Reineke, Williamson and Turner, 1941a). Graham Jnr., Jones and Kay (1936) reported that the A-V blood sugar difference was positively correlated both with the arterial blood sugar level and with the milk yield but these correlations were not confirmed by Shaw *et al.* (1938).

Qualitatively, these findings support earlier ideas regarding the formation of **lactose** from blood glucose, but the possibility that a proportion of the absorbed glucose may be oxidised or perhaps converted into milk fat must not be overlooked. Graham Jnr.'s (1937) quantitative arterio-venous studies in the goat, involving blood flow measurements, indicated that the glucose uptake was quite insufficient to account for the observed rate of lactose synthesis. The observation that considerable quantities of lactic acid were also utilised by the gland in these experiments suggested that this substance might also be used for lactose synthesis, though even then there was a deficit of about 15 per cent. of the lactose synthesis unaccounted for. Graham also observed a definite loss of amino-acids from the blood traversing the udder and he suggested that further lactose precursors might arise from deamination of amino-acids, presumably the anti-ketogenic moiety. The possibility that the mammary gland is capable of deaminising amino-acids is supported by the observations that the lactating udder in the goat produces urea (Graham Jnr., Houchin and Turner, 1937; Graham Jnr., Houchin, Peterson and Turner, 1938; Graham Jnr., Peterson, Houchin and Turner, 1938) and that actively lactating mammary tissue contains appreciable amounts of arginase (Shaw and Petersen, 1938b; Folley and Greenbaum, 1946, 1947). The uptake of glucose, lactic acid and amino-acids (calculated as alanine) determined by Graham Jnr. (1937) was together sufficient to account for the lactose synthesised, but Graham appears to have neglected the fact that part of the amino-acids, if indeed deamination does occur in the udder, would give rise to ketone bodies. Results confirming the uptake of lactic acid by the bovine mammary gland were obtained

by Shaw *et al.* (1938) who, however, found that the combined uptake of glucose and lactic acid was sufficient to account for the observed lactose synthesis. The position has since been complicated by the findings of Powell Jnr. and Shaw (1942) whose results indicated that lactic acid is absorbed by the udder from the blood only in cases of disturbance associated with the blood sampling. Later, Shaw *et al.* (1942) reported A-V blood sugar differences, in undisturbed cows, of sufficient magnitude by themselves to account for the observed rate of lactose synthesis provided of course that none of the sugar was used for energy production or conversion to fat. In this latter connection an additional source of carbohydrate for the mammary gland was indicated by Reineke, Williamson and Turner (1941b) who observed a definite uptake of glycoprotein, presumably globulin, by the udder of the anaesthetised lactating goat.

Studies of lactose synthesis *in vitro* have, on the whole, given results which are in harmony with the view that glucose is one of the main precursors of lactose. Early efforts to effect synthesis *in vitro* in the presence of mammary gland preparations were hardly convincing. Perhaps the first report of *in vitro* lactose synthesis to carry any degree of conviction was that of Michlin and Lewitow (1934) who used a mixture of glucose and galactose as substrate, but the first apparently unequivocal synthesis was achieved by Grant (1935) who obtained lactose synthesis by mammary gland slices from glucose but not from fructose, mannose or galactose. A mixture of glucose and galactose gave no more synthesis than glucose alone (Grant, 1936). Petersen and Shaw (1937) isolated lactose from a mixture of glucose and lactate incubated with macerated udder tissue, but in view of Grant's successful results with glucose alone this cannot be taken as evidence that lactic acid is an essential precursor of lactose. Weinbach (1936) claimed to have demonstrated the synthesis of a substance believed, perhaps on rather inadequate grounds, to be lactose, from glucose in the presence of a non-living preparation of mammary gland tissue. More recently Knodt and Petersen (1945), using mammary gland slices, have reported lactose formation in the presence of glucose, glucose and lactic acid, maltose and glycogen.

Additional evidence pointing to a relationship between blood sugar and lactose is provided by studies involving experimental alterations in the blood sugar level. Temporary lowering of the milk lactose content accompanies hypoglycaemia due to inanition (Nitzescu, 1925; Gowen and Tobey, 1931a), treatment with insulin (Nitzescu and Nicolau, 1924; Gowen and Tobey, 1931b; Brown *et al.* 1936a) or phlorizin (Paton and Cathcart, 1911; Gowen and Tobey, 1931b). Equally temporary and usually rather small increases in milk lactose content have been correlated with alimentary hyperglycaemia in cows (Whitnah, Riddell and Hodgson, 1933) and with prolonged hyperglycaemia caused by subcutaneous implantation of adrenal tablets in cows and goats (Bottomley, Folley, Walker and Scott Watson, 1939). On the other hand, hyperglycaemia produced in cows by infusion of glucose solutions into the udder (Brown *et al.*, 1936b) or into the external pudic artery (Petersen and Boyd, 1937) had no effect on the milk lactose.

The only firm conclusion that emerges from the sum total of the foregoing investigations is that glucose is almost certainly concerned in the synthesis of lactose and is probably its principal precursor. The afore-mentioned uncertainties connected with the quantitative aspects of arterio-venous studies together

with the paucity of well-attested knowledge of the oxidation processes occurring in mammary tissue and the fact that the ability of the udder to convert carbohydrate into fat is still controversial (*see* page 614) renders a decision as to whether other precursors are involved impossible at the present time.

Our knowledge of the intermediate stages in lactose synthesis is equally scanty. The possibility that glycogen is an intermediate, suggested by the presence of small quantities of glycogen in the mammary gland (Barrenscheen and Alders, 1932; Petersen and Shaw, 1938), has been strengthened by the finding (Knodt and Petersen, 1945) that perfusion of the isolated bovine udder with blood containing glucose markedly increases the glycogen content of the tissue. It is further significant that incubation of such glycogen-enriched udder tissue results in the disappearance of glycogen accompanied by the formation of lactose. As a result of perfusion experiments in which addition of insulin to the perfusion fluid increased the udder glycogen stores and decreased the lactose content of the milk secreted, Knodt and Petersen (1946b) postulated that lactose arises from the breakdown of glycogen. Phosphorylation processes as intermediate stages in lactose synthesis were postulated by Barrenscheen and Alders (1932) who, together with Borst (1932) and Brenner (1932), demonstrated the presence of phosphoric esters in the mammary gland. Grant (1936), however, found no evidence of increased lactose synthesis by mammary gland slices in the presence of hexose phosphates or phosphoglycerate. Lactic, pyruvic and citric acids—other possible intermediates—were found to have no effect on the ability of mammary gland slices to synthesise lactose (Knodt and Petersen, 1945). On incubation, mammary gland tissue produces lactic acid (Svanberg, 1930; Barrenscheen and Alders, 1932; Knodt and Petersen, 1945) and citric acid (Knodt and Petersen, 1946a); the production of the latter being increased in the presence of added glucose, lactic acid, pyruvic acid, maltose and glycogen.

Fat metabolism of the mammary gland.—In considering possible precursors of milk fat it seems *a priori* most likely that one fraction or a combination of fractions of the blood lipins—glycerides, phospholipins or cholesterol esters—is involved. The possibility that milk fat may, in part at any rate, be formed from carbohydrate, protein or amino-acids absorbed from the blood must, however, not be neglected.

Meigs *et al.* (1919), using the original Kaufmann-Magne method, found more lipin phosphorus in the jugular blood of lactating cows than in mammary venous blood, and accordingly advanced the theory that milk fat originated from the blood phosphatides. They considered that if this theory were true, the mammary gland would absorb more than sufficient lipin phosphorus to provide the phosphorus of the milk, and the return of the excess to the blood as inorganic phosphate would be expected. The results of Meigs *et al.* were apparently in accord with their theory in this respect since the mammary venous blood contained more inorganic phosphate than the jugular blood. This theory of the origin of the milk fat, which harmonised with prevailing views as to the prominent part played by phosphatides in intermediary fat metabolism was later upheld by Nikitin (1935) who also used the Kaufmann-Magne technique. Subsequent re-investigation of this question by workers using the more exact arterio-venous technique has, however, uniformly failed to demonstrate any uptake of blood phosphatide by the udder of the lactating cow (Blackwood, 1934; Graham Jnr., Jones and Kay, 1936; Maynard

et al. 1938) or goat (Lintzel, 1934). Moreover, far from the inorganic phosphorus content of the blood increasing as it passes through the lactating udder as demanded by the theory of Meigs *et al.*, workers using the arterio-venous technique have found that the active gland absorbs considerable amounts of inorganic phosphate from the blood (Blackwood, 1934 ; Lintzel, 1934 ; Graham Jnr., Jones and Kay, 1936). Lintzel (1934) and Graham Jnr., Jones and Kay (1936) were led by their results to the view that most of the milk phosphorus arises from the blood inorganic phosphate, a conclusion which has since received strong support from the results obtained by Aten Jnr. and Hevesy (1938) in a study involving the use of radioactive phosphorus. It may be concluded that at the present time there is no evidence for the participation of blood phospholipin in the formation of milk fat.

The currently accepted view that blood triglycerides are the precursors of milk fat was first advanced many years ago by Foà (1912) who perfused a sheep's udder with an emulsion of olive oil or triolein and observed the secretion of an aqueous fluid containing globules of fat, the iodine number of which was lower than that of the fat in the perfusion fluid. These results were regarded with scepticism by Meigs (1922) because the gland became oedematous during the perfusion. Later perfusion experiments by Petersen, Palmer and Eckles (1929) indicated that the mammary gland could absorb triglycerides, because after perfusion of an udder with an emulsion of corn oil stained with Sudan III, dye was found in the glandular tissues though none appeared in the secretion. It may be noted that a theory which postulates that the mammary alveolar cells are permeable to neutral fat does not conflict with modern views on the mechanism of fat absorption from the intestine (*see* Frazer, 1946).

Arterio-venous studies have on the whole given results which are consistent with the idea that the blood triglycerides are the main if not the sole precursors of milk fat. Lintzel (1934) in the goat, and Maynard *et al.* (1938) in the cow, obtained indirect evidence that the lactating udder selectively utilised the triglyceride fraction of the total blood lipins ; they observed a decrease in the total lipin of the blood passing through the gland but no evidence of any change in the phospholipin or cholesterol ester fractions. Similar results for the cow were reported by Graham Jnr., Jones and Kay (1936) except that these workers did not determine the cholesterol esters and thus did not exclude the possibility that fatty acids combined with cholesterol were also utilised by the gland. Subsequently, Voris, Ellis and Maynard (1940) devised a method for determining the blood triglycerides directly, depending on the estimation of glycerol after saponification of the acetone-soluble blood lipins, and were thus able to demonstrate the uptake of neutral fat by the lactating mammary gland more conclusively than had previously been possible.

In the cow, Shaw and Petersen (1938a, 1940) carried out arterio-venous studies in which blood fat was determined by a method which included cholesterol and cholesterol esters but not phosphatides and in which the blood/milk volume ratio was estimated from calcium uptake. Their results indicated that the blood fat uptake by the udder was more than sufficient to provide for the observed rate of milk fat formation and they therefore postulated that some blood fat was oxidised by the glandular tissues. The actual blood fat uptake would be even greater if, as Shaw and Petersen (1940) state, the udder produces a considerable flow of lymph containing much calcium but very little fat. Later, Shaw *et al.*

(1942) obtained results indicating the absorption of sufficient blood fat by the lactating bovine udder to provide the fat secreted in the milk. Shaw and Petersen (1940) reported that, contrary to what might be expected, there was no fat uptake by the udder immediately after milking; the uptake gradually rose during a few hours following milking and then remained sensibly constant for some time. The alveolar cells were thought to be permeable to blood fat only when the alveoli were distended with milk.

The possibility that a portion of the milk fat is synthesised from carbohydrate was suggested by Graham Jnr., Houchin, Peterson and Turner (1938) because they obtained a mean respiratory quotient (R.Q.) greater than unity for the udder of the lactating goat. This finding was confirmed for the goat by Reineke, Stonecipher and Turner (1941) who obtained a mean value of 1.18 for the R.Q. of the udder of the anaesthetised goat, after application of a correction for carbon dioxide supposedly used for the synthesis of urea by the udder. This correction was made on the assumption that the urea is formed from carbon dioxide and ammonia arising from the deamination of amino-acids as suggested by Graham Jnr., Houchin and Turner (1937) and not from the decomposition of arginine absorbed from the blood by the mammary gland. Bottomley and Folley (unpublished observations) in a series of determinations of the mammary gland R.Q. in lactating cows found no such variability as was reported by Shaw (1939) and Petersen and Shaw (1942) in cows and Reineke, Stonecipher and Turner (1941) in unanaesthetised goats. Their value for the mean R.Q. of the lactating bovine udder in cows, in experiments in which there was no haemoconcentration, was 1.25 ± 0.12 (5 per cent. fiducial points); in experiments in which haemoconcentration occurred the R.Q. was, however, less than unity. Shaw (1942b) obtained an R.Q. of 1.15 for the udder of the cow in ketosis. Mammary gland R.Q. values below unity have been reported for perfused bovine glands by Shaw (1939), for fasted lactating and non-pregnant dry goats by Reineke, Stonecipher and Turner (1941) and for fasted lactating cows by Shaw *et al.* (1942). Reineke, Stonecipher and Turner (1941), however, question whether values obtained on perfused glands are relevant to the intact gland *in vivo*. The consensus of evidence undoubtedly points to an R.Q. greater than unity for the lactating udder in the intact animal, but it must be conceded that the R.Q. may be a misleading index of reactions occurring even in a single organ so that further independent evidence would seem to be required before the formation of fat from carbohydrate in the mammary gland can be taken as established (*see* page 615).

The origin of the short-chain fatty acids which are a striking feature of the milk fat in the ruminant (*see* page 598) is an intriguing problem which has not yet been satisfactorily solved. The main possibilities as to their origin may be summarised thus: (a) they may be formed in the gland from long-chain fatty acids by a process of oxidation followed by reduction; (b) carbohydrate absorbed from the blood by the gland may be there transformed into short-chain fatty acids; (c) they may be formed in the gland by the transformation of, or synthesis from, lower fatty acids arising in the course of metabolism and transported to the gland by the blood. These possibilities will now be considered in turn.

(a) The possibility that the short-chain fatty acids of milk fat are formed from oleo-glycerides by oxidation in such a manner that the carboxyl groups are not involved and that one molecule of oleic acid gives rise to one molecule of short-

chain fatty acid was suggested by Hilditch and his colleagues (Hilditch and Sleight-holme, 1931; Hilditch and Paul, 1936; Hilditch and Thompson, 1936) in consequence of regular relationships which emerged in a study of the fatty acids of the component glycerides of bovine milk and depot fats. This theory was supported by the fact that in the lowest unsaturated fatty acids found in milk fat the double bond is in the same position relative to the carboxyl group as in oleic acid (Hilditch and Paul, 1936) suggesting that these acids represent intermediate stages in the degradation of the carbon chain of oleic acid and by the observation that the feeding of cod-liver oil results in a reduction of the proportion of short-chain acids and an increase in the proportion of oleic acid in the milk fat glycerides (Hilditch and Thompson, 1936). This, it was believed, was due to the poisoning by highly unsaturated acids present in cod-liver oil of an oxidising enzyme system responsible for the degradation of the oleic acid chain. Hilditch (1937) in summarising the evidence for his theory points out that it is in accord with modern views on the mechanism of oxidation of long carbon chains exemplified by the alternate multiple oxidation theory of Jowett and Quastel (1935) and the ω oxidation theory of Verkade and van der Lee (1934). The results of Smith and Dastur (1938), who found that during inanition the short-chain fatty acids of bovine milk fat decrease concomitantly with an increase in the proportion of oleic acid, are also in harmony with this theory. Shaw and Petersen (1938a) who, as we have seen, reported that the lactating bovine udder absorbs more than sufficient fat to provide for the observed rate of fat synthesis, also postulated that fatty acids were partially oxidised in the mammary gland, thus giving rise to the short-chain fatty acids of milk. In accordance with this view, Shaw (1939) obtained an R.Q. of 0.8 for perfused lactating bovine udders which was taken to be indicative of fat oxidation. However, it seems probable that the R.Q. for the intact udder is greater than unity and the R.Q. in any case cannot be relied on as an indicator of the reactions occurring in an organ with so complicated a metabolism as that of the mammary gland.

(b) The evidence in favour of the possibility that the milk short-chain fatty acids are synthesised from carbohydrate consists in the observation that the R.Q. of the intact lactating gland is greater than unity and declines below unity during inanition (*see* page 614). Since inanition also lowers the proportion of short-chain acids in the milk triglycerides (e.g. *see* Smith and Dastur, 1938) it seems possible that if the high R.Q. of the normal gland does denote the synthesis of some portion of the milk fat from carbohydrate, then this portion may consist particularly of the short-chain fatty acids as suggested by Reineke, Stonecipher and Turner (1941). Smith and Dastur (1938) suggested that their results, already mentioned under (a) above, would be in accord with this hypothesis if it be assumed that oleic acid is synthesised in the mammary gland from carbohydrate, the short-chain acids representing by-products of this process. The decline in the latter during inanition, they say, could then be ascribed to the greater facility with which oleic acid synthesis would go to completion in conditions such as those of inanition, in which the total production of milk fat declines. On this view, however, the mammary gland R.Q. during inanition should be greater than unity, since oleic acid is still being synthesised from carbohydrate, whereas Shaw *et al.* (1942) found values less than unity under these conditions. Moreover, Hilditch, Paul, Gunde and Maddison (1940) believe that the glyceride

structure characteristic of milk fat is such as would be unlikely to result from a process of fat formation from carbohydrate.

(c) The possibility that the short-chain fatty acids of milk fat pre-exist in the blood has no evidence to support it. Such a mixture of fatty acids has never been detected among the blood lipins (*see* Smith and Dastur, 1938). However, the observation of Shaw and Knodt (1941a) that the lactating bovine udder absorbs considerable amounts of β -hydroxybutyric acid from the blood led them to suggest that the short-chain fatty acids of milk might be elaborated from this blood constituent. The observed uptake was sufficient to account for the milk lower fatty acids up to those with chains of fourteen carbon atoms. Such a synthesis would, of course, tend to increase the R.Q. and its occurrence would thus be consistent with experimental findings. Further support to this theory of the origin of the lower fatty acids of milk came from further observations of Shaw and Knodt (1941b) who reported a decrease in the mammary gland R.Q. as well as in the milk short-chain fatty acids in cows fasted or fed cod-liver oil. However, since the short-chain acids in the milk fat undergo a decrease in cows suffering from severe ketosis (Shaw, 1941; Shaw *et al.* 1942) while the mammary gland absorption of β -hydroxybutyric acid is markedly increased (Shaw, 1942b), it seems more likely that this intermediate of fat metabolism is oxidised in the udder as suggested by Shaw (1942b), Shaw *et al.* (1942) and Shaw and Petersen (1943).

There remains another and most interesting possibility which at present is purely theoretical.* It must surely be regarded as significant that short-chain fatty acids are, as far as present knowledge goes, only found in any quantity in the milk of ruminants (*see* page 598). It has recently been shown that in ruminants considerable amounts of lower fatty acids, particularly acetic acid, are produced as a result of the degradation of carbohydrate by the micro-organisms of the rumen and are absorbed into the blood (*see* Elsdon and Phillipson, 1948). Moreover, Rittenberg and Bloch (1945) using acetic acid marked with heavy carbon have demonstrated the synthesis of carbon chains from acetic acid in the body. It therefore seems possible that the lower fatty acids of milk fat may be synthesised in the udder from acetic acid absorbed from the rumen and transported to the udder by the blood. Such a reaction might explain the high R.Q. of the intact lactating gland since it involves the synthesis of oxygen-poor compounds from a compound relatively rich in oxygen.

Nitrogen metabolism of the mammary gland.—Relatively little is known about the nitrogen metabolism of the mammary gland. It is clear that some fraction or combination of fractions of the nitrogenous compounds of the blood must provide the precursors of the milk protein, the most likely *a priori* possibilities in this connection being the amino-acids and some fraction of the blood proteins.

Cary (1920) using the Kaufmann-Magne technique concluded that the blood amino nitrogen suffered a diminution in passing through the udder of the lactating but not the dry cow. He was thus led to the view that the milk proteins are synthesised from the amino-acids of the blood. This theory seemed to be supported by subsequent arterio-venous studies which have uniformly demonstrated a small but definite uptake (in most cases less than 1 mg. amino N/100 ml.) of

* Since this chapter was completed in 1945 evidence has come both from *in vitro* experiments on mammary gland slices (Folley and French, 1950), and from an *in vivo* experiment on a lactating goat (Popják, French and Folley, 1950) that acetate is utilised for milk fatty acid synthesis by the mammary gland. This and other aspects of the biochemistry of lactation have been reviewed by Folley (1949).

amino-acids by the lactating udder of the cow (Blackwood, 1932 ; Shaw and Petersen, 1938a, c ; Bottomley and Folley, unpublished observations) and goat (Lintzel, 1934 ; Graham Jnr., 1937 ; Graham Jnr., Peterson, Houchin and Turner, 1938 ; Reineke, Peterson, Houchin and Turner, 1939), but not by the non-lactating bovine udder (Blackwood, 1932). Quantitative arterio-venous studies have, however, indicated that the uptake of amino-acids from the blood is quite insufficient to provide the proteins of milk (Graham Jnr., 1937 ; Shaw and Petersen, 1938a, c ; Reineke *et al.* 1939), the observed A-V differences corresponding to only about 40 per cent. of the milk nitrogen. The conclusion therefore seems inescapable that if the blood amino-acids are concerned in the formation of milk proteins, their rôle is inconsiderable. Further evidence against Cary's theory was cited by Reineke, Williamson and Turner (1941b) who could detect no uptake of amino nitrogen by the udder of the fasted goat even though milk secretion continued.

Graham Jnr., Peterson, Houchin and Turner (1938) suggested that blood globulin may be utilised by the mammary gland for transformation into milk protein. They found that the udder of the lactating goat absorbed from the blood, in addition to amino-acids, appreciable quantities of other non-protein nitrogenous substances and of a fraction of the blood protein identified as globulin, while returning albumin to the blood. They suggested that the globulin molecule might undergo partial degradation in the mammary gland into still relatively large molecular fragments some of which might be used, together with non-protein nitrogenous material from the blood, for the synthesis of milk protein, the residues being returned to the blood stream. Later, rather irregular changes in various fractions of the blood nitrogen passing through the mammary gland were reported by Reineke *et al.* (1939). These results seem hardly susceptible of a simple interpretation, but the authors suggest that they are consistent with the idea that plasma protein fractions undergo reversible shifts among themselves in the mammary gland involving partial breakdown of the molecules as suggested by Graham Jnr., Peterson, Houchin and Turner (1938). The possibility that the mammary gland may at some periods in the lactation cycle store nitrogenous substances which later may be partially utilised for the synthesis of milk protein may be offered towards an explanation of such results which are otherwise difficult to interpret. Further evidence that blood globulin may be a precursor of milk protein has been provided by Reineke, Williamson and Turner (1941b) who observed the uptake of glycoprotein, believed to be globulin, by the lactating udder of the goat.

It is not certain whether all or part of the blood amino-acids absorbed by the lactating mammary gland are incorporated into the milk protein or whether some proportion is deaminised. An appreciable output of urea by the goat udder has been reported (Graham Jnr., Houchin and Turner, 1937 ; Graham Jnr., Peterson, Houchin and Turner, 1938 ; Reineke *et al.* 1939) and it has been claimed that in early lactation the output of urea nitrogen exceeds the intake of amino nitrogen (Graham Jnr., Peterson, Houchin and Turner, 1938). It seems possible that some of the urea could arise from the decomposition of arginine by the action of the arginase known to be present in mammary tissue (Shaw and Petersen, 1938b ; Folley and Greenbaum, 1946, 1947).

The unidentified portion of the plasma non-protein nitrogen which, according to Reineke *et al.* (1939) is absorbed by the lactating goat udder may also be concerned in the elaboration of milk protein. The studies of Shaw and Petersen (1938c),

however, indicate that uric acid, creatine and creatinine are not involved since these blood constituents were not utilised in significant amounts by the udder of the cow.

The phosphorus of casein appears to come from the inorganic phosphate of the blood (Aten Jnr. and Hevesy, 1938).

Enzymes of the mammary gland.—The enzymes of the mammary gland have been relatively little studied, despite the interest of the subject as affording an insight into the chemistry of milk synthesis.

The most closely studied enzyme of mammary tissue is the alkaline phosphatase first detected in the mammary gland by Kay (1925). In the same year Tateyama (1925) obtained evidence of the presence in mammary gland extracts of an enzyme capable of dephosphorylating nucleic acid and hexose phosphates. Later, Borst (1932) confirmed the presence of a phosphatase in mammary gland tissue. Folley and Kay (1935) examined many of the properties of the alkaline phosphatase (optimum pH 9-10) of the guinea-pig mammary gland and showed that in all respects studied it was identical with the phosphatase of the kidney and therefore to be classed as a phosphomonoesterase A₁ in the classification of phosphatases proposed by them (Folley and Kay, 1936). Caputto and Marsal (1941) have described a method of purification of mammary gland phosphatase and on the basis of studies of its inhibition by various ions (Caputto and Marsal, 1944a), believe it to be identical with the alkaline phosphatase of bone, intestinal mucosa and blood plasma, but different from that of the kidney. Spectrographic examination of purified preparations (Caputto and Marsal, 1944b) indicate the presence of a protein rich in aromatic amino-acids. Folley and Kay (1935) found that the phosphatase potency of lactating mammary tissue was of the same order as that of kidney tissue so that the mammary gland is one of the three or four richest known sources of the enzyme. More extensive studies in the lactating rat by Folley and Greenbaum (1946, 1947) indicate that in this species mammary tissue exhibits about 33 per cent. of the phosphatase activity of the kidney. Neither adrenalectomy nor treatment with adrenal cortex steroids significantly altered the phosphatase levels of the rat mammary gland.

Arginase was detected in lactating bovine udder tissue by Shaw and Petersen (1938b) who could find none in non-lactating tissue. The presence of arginase in the lactating mammary gland of the rat has recently been reported by Folley and Greenbaum (1946) who find the arginase potency of mammary tissue to be about 2.5 per cent. of that of the liver. The former may thus be reckoned as the second most potent source of arginase in the body. A study of the mammary gland arginase during pregnancy, lactation and involution in the rat showed that the mammary gland arginase content increased only slightly during pregnancy and during early lactation but underwent a dramatic increase in mid-lactation. By contrast the alkaline phosphatase increased during the mammary growth phase and remained at a high but constant level throughout lactation (Folley and Greenbaum, 1947). The arginase content of the mammary gland decreased significantly after adrenalectomy (Folley and Greenbaum, 1946) but was not restored to normal levels by treatment with adrenal cortex steroids.

Other enzymes which have been reported as present in the mammary gland tissue include lipases (Tateyama, 1925; Virtanen, 1924; Kelly, 1938), amylases (Grimmer, 1913; Tateyama, 1925), maltase (Kleiner and Tauber, 1932) and purine oxidases (Michlin and Ryżowa, 1934).

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CHAPTER 21

FERTILITY

By JOHN HAMMOND

I. INTRODUCTION

Problems of the control of fertility and sterility are of great practical importance. The rate of reproduction in man and methods of controlling it have been, and are, a matter of concern to the statesmen of different countries; the increase in fertility and elimination of sterility from our studs, flocks and herds is of enormous importance to the livestock industry and food supplies (*see* Heape, 1906), while a knowledge of the methods by which reproduction can be controlled may assist in the limitation of plagues of insects and rodents and other pests.

The rate of propagation in any species of animal depends not only on the average number of young born in each litter (*see* Asdell, 1946), but also on the frequency of recurrence of the sexual season (*see* Chapters 3 and 4), on the duration of pregnancy (*see* Chapter 19) and the suckling period, and on the duration of the reproductive period in the animal's life. The rate of increase of any species also depends on the age at which puberty is attained, and this as a general rule is earlier in small species than in large ones. In the male the sexual maturity is usually reached later than in the female, but there is no definite ending of the reproductive period as there is with the female in some species, such as man, in which the climacteric marks the cessation of generative activity.

There are several broad generalisations which may be made with regard to fertility, which although true in the main, have to be qualified when the details are considered, as they will be below. Broadly speaking, the average number of young produced in a litter in any species of mammal is inversely proportional to the average size of the animals belonging to that species; thus the large cow but rarely produces twins, whereas the small rat occasionally bears as many as sixteen or even twenty young. This, however, does not hold for the size of the breed within one species (*see* below). Another exception to this general statement is to be found in the bats, which are remarkable for their relative infertility, only one young ordinarily being produced at a time. Herbert Spencer (1899) elaborated a theory of fertility which may explain this exception. He suggested that the power to sustain individual life and the power to produce new individuals are inversely proportional, that is, that Individuation and Genesis vary inversely. Where there is abundant food supply and a favourable environment, and the necessary expenditure of energy is relatively slight, the cost of Individuation is much reduced and the rate of Genesis is correspondingly increased. Thus he ascribes the low fertility of the bat, as compared with the mouse of much the same size, to the relatively high rate of expenditure resulting from the habit of flying.

He contrasts the relative fertility of birds and mammals in the same way. Predatory birds have fewer young ones than predatory mammals of approximately the same sizes, and, moreover, in the case of birds which fly much both parents have to help to rear the young, whereas in mammals the mother is able unaided to suckle and rear the litter.

There can be little doubt that Spencer's generalisation is in the main true, but it is equally certain that it cannot be applied to explain the relative degrees of fertility in all animals. Generally speaking, only one young is produced in those animals in which the period of gestation exceeds six months; perhaps this is because it is generally the larger species of animals which have the longer gestation periods. The number of teats characteristic of the species also affords an approximate indication of the average size of the litter. This is probably a correlation produced by evolution rather than a physiological correlation, for under domestication and selection for one character without the other a divergence may be produced between the two. For example, many individuals of the improved breeds of pigs such as the Middle White, which have been selected for fertility, may produce as many as 17 young although they may have as few as 10 teats. Moreover, in Bell's flock of sheep which were selected for high teat number (as many as 8 instead of the normal 2 having been obtained) Castle (1924) could find no evidence for any relation between teat number and multiple births. In *Dasyurus*, too, as Hill (1910) points out, the number of eggs shed, up to 35, is far in excess of the teat number, usually 6; similarly Hartman (1929) in the opossum has found up to 56 eggs in the uteri.

While there are many facts concerning fertility and sterility which cannot at present be explained by existing knowledge of the physiology of reproduction, there are several which can be assigned to different phases of the reproductive process. Many of what were hitherto detached facts concerning fertility can now, by the advances made in the physiology of reproduction in recent years, be brought together and grouped according to the causal factor concerned. As many as possible of the facts and theories concerning fertility will therefore be considered below under the different phases of the reproductive process at which they take effect, for it is at these phases of the process: the number of the ova shed (II); the number of ova fertilised (III); and the number of embryos developing to birth (IV), that we may hope to obtain a measure of control over fertility.

II. THE NUMBER OF OVA SHED

It has long been known that the number of primordial ova existing in the ovaries of a mammal greatly exceed those which ever have a chance of being fertilised during the life of the animal. Thus the human ovary is said to contain 20,000 oöcytes at puberty, or sufficient to admit of 40 ova being discharged every month throughout the reproductive period of life. In the rat there are, according to Arai (1920a), 35,100 ova at birth, but these are reduced by degeneration to 11,000 after 23 days and 6,000 by the 63rd day.

Those who first investigated the problems of differences in fertility between different breeds and individuals of domestic animals naturally looked to see if they could be accounted for by differences in the number of ova contained in the

ovaries, but found none. Pearl (1912), for instance, observed no correlation between the number of visible oöcytes in the ovary and the egg production of fowls. Differences are found, however, when only large follicles which can be observed by eye are taken into account. Thus Käppeli (1908) found in the wild boar, which usually produces only one litter of 3 to 8 a year in February or March, an average of 24 follicles over 3 mm. diameter in the ovaries, whereas in domestic pigs, which may produce litters of 12 or more twice a year, he found an average of 46 follicles of this size in the ovaries. He observed that the difference between the high fertility type and the low fertility type was highest (335 per cent.) among the large follicles over 5 mm. diameter, and lowest among the small follicles of 3 mm. diameter (66 per cent.).

In the main it is not the number of oöcytes present in the ovary, but the number of follicles which ripen at each oestrus which determines the potential fertility of the female. Cases, however, have been found in which more than one ovum is contained within a follicle. Polyovular follicles have been described in the guinea-pig (Loeb, 1917), opossum (Hartman, 1926, who gives other references), mouse (Engle, 1927b), and other species, but there is at present little or no evidence that these play any part in increasing fertility in the individual. Rather, their occurrence has been attributed (Loeb, 1917) to malnutrition of the ovary, but Allen *et al.* (1947) have estimated that in the wild rabbit 0.23 per cent. of the follicles which ovulate produce two embryos through the liberation of two ova.

That the number of follicles which ripen at any one oestrus is due to the presence of certain substances in the blood stream, and not to the ovary itself, was suggested by the experiments of Arai (1920b), Slonaker (1927), Asdell (1924), Lipschütz (1927), and others, who found that a similar number of follicles were ripened by the individual whether two, one, or only part of one ovary was left in the animal. On the strength of these and other experiments, Hammond and Marshall (1925) adopted the idea of Heape (1905) that a substance external to the ovary and circulating in the blood (called by him "generative ferment"), formed the basis for ovarian activity. As was shown later by Smith and Engle (1927), Zondek and Aschheim (1927) and others, this substance was the gonadotrophic principle of the anterior pituitary gland (*see* Chapter 12).

Gonadotrophic Hormones

Although a vast number of papers have been published on the gonadotrophic hormones of the anterior pituitary, they have been concerned for the most part with the separation of these hormones into follicle-stimulating (F.S.H.) and ovulating or lutealising (L.H.) fractions and other aspects of purely theoretical interest. This also applies to the other gonadotrophic hormones such as the lutealising hormone obtained from the urine of pregnant women (P.U.) and the follicle-stimulating hormone which is found in the blood serum of the mare between about the 45th and 100th day of pregnancy (P.M.S.). It is only comparatively recently, however, that attempts have been made to use these hormones to increase the fertility of animals.

Numerous workers have shown how, by injections of these hormones, the number of ova shed may be considerably increased (Engle, 1927a, 1931, Bel-

lerby, 1929, and others). Priming doses of F.S.H. followed by ovulating injections of L.H. will cause the shedding of up to 80 eggs in the rabbit (Pincus, 1940).

Knowing the effects of these hormones on the ovary it might seem to be a simple matter to increase the fertility of an individual by injection, but this is not the case. For example, Zawadowsky and Padootcheva (1935) found that they could cause superovulation by injections of 150–500 m.u. of pregnant women's urine hormone into ewes either 1–4 days before, during, or 2–3 days after oestrus, but in no case was more than one lamb produced. Lopyrin (1937, 1938), by injecting 100–140 m.u. of pregnant women's urine hormone into ewes 2–4 days before oestrus, was able to obtain superovulation, in most cases of 3–4 follicles, and in some cases up to 7–8 follicles. The lambing percentage was, however, only 20–60 per cent. higher than the controls and a high proportion of the ewes failed to conceive at all. As is pointed out below, failures in such cases may be due to lack of fertilisation caused by faulty timing of ovulation in relation to mating (p. 681) or to lack of implantation due to lack of synchronisation between the development of the blastocyst and the development of the corpus luteum (p. 700). Better results were obtained, however, by Loginova and Lopyrin (1938) and by Zawadowsky (1941) by making two injections of pregnant mare serum hormone, the first on the 14th day after the previous oestrus (the ewe having a 16-day cycle), and the second at the onset of oestrus. Multiple ovulations were caused and fertility was considerably increased, quadruplets and quintuplets being born.

The number of ovulations which can be obtained from a given dose of follicle-stimulating hormone is determined by conditions which are not yet fully understood. In the rabbit, a species which requires the stimulus of coitus acting on the anterior pituitary gland to set free the lutealising hormone causing ovulation, multiple ovulations can easily be caused by three small daily doses of follicle-stimulating hormone followed on the 4th day by intravenous injection of lutealising hormone (Pincus, 1939, 1940; Parkes, 1943). In the rat, a species in which the influence of the corpus luteum on the cycle is comparatively insignificant, it is only in immature animals that superovulation is caused by injection of gonadotrophic hormones (Cole, 1937); in these up to 27 young were found in the uterus when the mothers were killed on the 17th–22nd days of pregnancy, while up to 17 per litter were born alive. Evans and Simpson (1940) have shown, too, in rats, that superovulation by injections of F.S.H. may be followed by the implantation of many such ova without increase in the number of young born owing to foetal atrophy (*see* p. 705).

In the sheep during the anoestrous period, when no corpus luteum exists in the ovary, injection of follicle-stimulating hormone (horse pituitary) causes the ripening of large numbers of follicles, the exact number depending on the dose, but results in the ovulation of only the normal number for the species. This is presumably because the rupture of the ripest follicle or follicles inhibits the rupture of the others, for the same amount of follicle-stimulating hormone gives very different results according to the time during the oestrous cycle at which it is injected. If injections are made 4 days after oestrus, when the corpus luteum is young and growing, no ovulation is obtained; if injection is made, however, at about the 13th day of the cycle, when the corpus luteum is beginning to wane,

multiple ovulations of up to 7 ova are obtained at the subsequent oestrus on the 16th day. It would appear that the existence of a corpus luteum is sufficient to prevent immediate ovulation of the first ripening follicle and gives time for many follicles to become mature before the trigger for ovulation (the waning of the corpus luteum) is released (Hammond, Jnr., Hammond and Parkes, 1942). Up to 15 fertilised eggs have been recovered from the tubes at 2 days, and 5 embryos from the uterus at the 3rd month have been recovered from ewes treated in this way (Hammond, 1940).

Casida *et al.* (1944a) who superovulated ewes by 4 consecutive daily, subcutaneous injections of F.S.H. of sheep's pituitary beginning on the 12th day of the cycle followed by intravenous injection of L.H. from the same material on the 6th day after the first F.S.H. injection, obtained an average of 9.2 eggs fertilised with 22 corpora lutea in the ovaries. The ewes were allowed to mate naturally, or artificially inseminated on the day of the intravenous injection and also on the following day. While there was no doubt about the initial fertility, 70 per cent. of a total of 81 eggs were cleaving when washed out 2 to 5 days following insemination, by the 14th to 27th day an average of only 3.4 embryos remained, and by the 30th to 37th day an average of only 0.8 embryos remained. Two possible causes of death are suggested: (1) inadequate uterine nutrition due to direct or indirect methods of treatment, or (2) abnormalities induced in the ova by rapid maturation of follicles. They attempted to test this by transplanting some of the ova into normal ewes, but failed owing to technical difficulties in transplantation (*see also* p. 702).

The cow behaves in much the same way as the sheep. After the injection of P.M.S. into the cow, a large number (according to the dose of P.M.S.) of follicles will ripen, but whether one or more will ovulate or not depends on the presence or absence of an active corpus luteum in the ovary. If an active corpus luteum is present in the ovary, then a large number of follicles will ripen and become big, and often very big, cysts, but no ovulation will occur (Folley and Malpress, 1944). If, however, the active corpus luteum is then expressed from the ovary, ovulation will shortly occur of all those follicles which have attained maturity (there may be 20 or more depending on dose and time since the injection of P.M.S.) (Hammond, Jnr., and Bhattacharya, 1944). If, on the other hand, no corpus luteum is present in the ovary at the time of injection of P.M.S., the first follicle which attains maturity ovulates spontaneously and the formation of the new corpus luteum inhibits ovulation of the other follicles so that only single ovulations occur in spite of large doses of P.M.S. Multiple ovulations in the cow can also be produced by making the injection of F.S.H. some 3 or 4 days before the natural oestrus is due, i.e. at a time when the activity of the corpus luteum is naturally on the wane. Attempts are now being made to use this in commercial beef production (Fig. 21. 1) so as to obtain twins and triplets in cattle (Zawadowsky (M.), 1941; Hammond, 1946a). In cases where more than about 3 ova are fertilised the additional embryos atrophy (*see* p. 705). About 2,000 i.u. are required to produce twins or triplets in cattle.

Instead of injecting subcutaneously a gonadotrophic hormone like P.M.S., which largely consists of F.S.H., and relying on the cow's own pituitary to supply sufficient L.H., a substance which contains both F.S.H. and L.H. in considerable quantities (such as sheep's pituitaries) may be used to cause multiple ovulation.

Subcutaneous injections are first given and these cause follicular growth, while 4 days later an intravenous injection is given and this causes ovulation, F.S.H. being most effective when given subcutaneously and L.H. when given intravenously (Pincus, 1946).

According to Zawadowsky (B.) (1945), different samples of P.M.S. vary greatly in the proportions of F.S.H. and L.H. that they contain ; for obtaining ovulation and progeny he found that a ratio of 4 F.S.H. to 1 L.H. (in m.u.) gives the best results.

The effect of gonadotrophic hormone injections in the mare is quite different from those in cattle and sheep in that they cause little or no follicular growth, and



Fig. 1. Shorthorn dam and her three-month-old triplet calves, born as a result of P.M.S. injections before service.

much the same occurs in woman, both these species producing gonadotrophic hormones during pregnancy.

In species like the rabbit which only ovulate after coitus, the orgasm releasing L.H. from the pituitary, sterility is sometimes caused by failure to ovulate after mating. This probably accounts for the low percentage of fertility in the Flemish breed (Wilson, 1940). Intravenous injection of 20 to 40 m.u. of P.U. at the time of mating will remedy this (Padootcheva *et al.*, 1935) (*see further* p. 666). In the vole (*Microtus*), which like the rabbit ovulates only after coitus, ingestion as well as injection of gonadotrophins causes increase of litter size, and Bodenheimer and Sulman (1946) suggest that it is the ingestion of plant gonadotrophins which cause the periodic vole outbreaks in nature. This was also suggested by Heape (1931) to account for the rapid multiplication and migration of the lemming in certain years.

The discovery of anti-gonadotrophic hormones (Parkes and Rowlands, 1936, Collip, 1938, Collip *et al.*, 1940) in recent years opens up possibilities for limiting fertility (*see* Chapter 12) but the practical applications of these have not yet been fully explored.

Age

The effect of age on the number of ova shed may in general be described as a wave which, starting from sterility, rises somewhat rapidly to its highest point and then gradually falls again to sterility.

Pearl (1913) refers to the case of a ewe whose complete breeding record conforms to the general age curve. In her first two years she produced singles, in her third year twins, then for six years in succession triplets, for the next six years twins and finally for two more seasons singles. In the next two years, which were the last of her life, this ewe did not produce any lambs. In most animals the first litters are smaller than successive ones, while in the declining years of life there is a diminution until sterility is reached once more. This is said to be the case with the bear, the elk and other animals (Duncan, 1866). A similar general rule holds good for cattle, sheep and pigs (Jones and Rouse, 1920; Harrison, 1916).

Many investigations have been made to determine in different species the exact age of puberty, maximum fertility and the time of decline in ova production in old age, and a few examples of the age of maximum fertility may be quoted. Facts concerning the age of puberty and old age will be found in Chapter 23.

The shape of this egg production and fertility curve naturally varies in different species, and, in domestic fowls, also with the breed. While in unimproved breeds of fowls maturity may be late and the maximum egg production may not occur until the second or even third year (Geyelin, 1865), in the modern improved breeds the greatest egg production occurs in the first pullet year, calculating this from October 1 after the date of hatching, or in the second year of life calculated from the date of hatching (Pearl, 1912, 1917). Ball, Adler and Egbert (1916) found the average egg production per hen for each year of life in their investigations on White Leghorns to be as follows:—

Year of life	.	.	1	2	3	4	5	6	7	8
No. of eggs per year	.	.	130	120	110	85	75	65	55	45

Similar results were obtained by Brody *et al.* (1938) and Brody (1945).

In the Copenhagen (1914) egg-laying trials it was found that the older hens, as compared with the younger, lay a smaller percentage of their eggs during the winter months. This has been confirmed by Zander *et al.* (1942), who conclude that the major factor involved in the decline of egg production with age is in the length of the laying year, with the decline in general rate making a small but significant contribution.

The size of the litters has been observed to increase up to 16 months in guinea-pigs (Minot, 1891); to the second litter (Crampe, 1883), 7 months (King, 1916), or 150–180 days (Feldman, 1926) in rats; to the second litter in mice (Parkes, 1924); and to the fourth (Johannson, 1929), fifth (Yamane and Makita, 1934; Olbrycht, 1943), or sixth (Zorn, Krallinger and Schott, 1933) litter in pigs. Krizenecky (1935b, 1936) finds that in pigs the relation between litter size and litter number is affected by selection in later litters and that maximum litter size is reached with the second litter. Stewart (1945a) finds that gilts farrowing for the first time at 320 days old average one pig less, and those farrowing at 410 days average half a pig more, than those farrowing at 365 days old. Lush and Molln (1942) from extensive data conclude that the number of pigs farrowed rises to

about 2 years old, remains at high level till about $4\frac{1}{2}$ years old after which it slowly declines again. Nichols (1926a) found that young ewes pregnant for the first time (18 months) gave a much smaller lambing percentage than older ewes, while Wriedt (1925) observed that fertility rose in sheep up to the fifth year. Kelley (1939) has shown that in Merino ewes the percentage of twins rises to a maximum between 5 and 10 years old and then falls to a low level in later years (Fig. 21. 2). There are differences, however, due to breed variations ; while in early

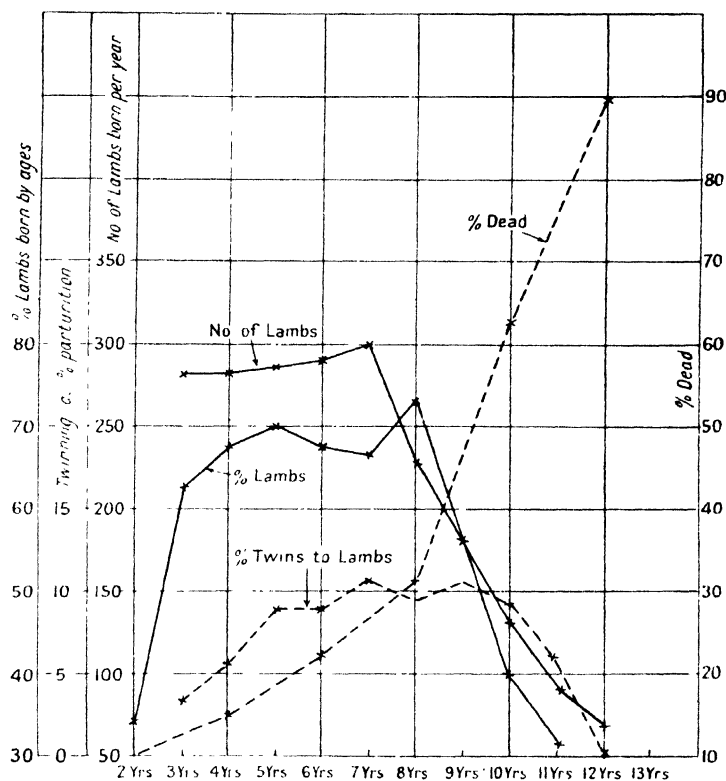


Fig. 21. 2 -Age changes in fertility, twinning and mortality in a flock of Merino ewes. (From Kelley, 1939.)

maturing breeds of sheep the maximum fertility may be attained at 3 years old, in late maturing breeds it is not attained until 5-6 years old (Lopyrin, 1938).

The causes for the lack of ova production in early life are not due to the age of the ovarian tissue itself, for Foà (1901) found by reciprocal transplantation of mature and young ovaries into young and mature rats that it was the age of the animal and not that of the ovary which determined the ripening of follicles. Later experiments by Zondek and Aschheim (1927), Smith and Engle (1927) and others, who transplanted or injected extracts of anterior pituitary glands, showed that immature animals could be made to shed their eggs by this means. It can therefore be concluded that the blood of immature animals is lacking in a

sufficiency of the gonadotrophic substances of the anterior pituitary to cause ovulation. Where this is supplied by injection immature ovaries can be made to shed more ova than adult ovaries (Cole, 1937).

Even after the onset of puberty is reached there is generally a period of time elapsing before the maximum number of ova are shed at ovulation. Hammond (1914) found that whereas young growing sows only averaged 14 corpora lutea in the ovaries, adult fully grown sows averaged 20. MacDowell and Lord (1925) also obtained similar results with mice, the rise in number of ovulations being especially rapid for the early litters. In general, the time of submaximal fertility corresponds to the time during which the skeleton is completing its full growth, and it therefore seems probable that some differential production between the growth hormone and gonadotrophic hormones of the anterior pituitary is involved; little exact information, however, is yet available on this point.

There is at present a division of opinion as to whether the prepubertal anterior pituitary contains less gonadotrophic hormone than the adult gland; Lipschütz and Reyes (1932) found that there is less relative to weight in adult glands, while Wells (1938) finds that the potency of the prepubertal glands is less than that of adults (*see* Chapter 12). In young (4-6 months) rabbits, Pincus (1940) found that copulation alone (releasing L.H. from the pituitary) cannot substitute P.U. in causing superovulation after priming doses of F.S.H.

There is apparently an optimum age at which to breed young animals for the first time. If mating takes place too early a smaller number of ova will be shed, possibly owing to subnormal F.S.H. production. On the other hand, if the first mating occurs late in life there appears to be greater development of the individual in general at the expense of fertility (Spencer, 1899), this possibly being due to interactions between the gonadotrophic and growth hormones of the anterior pituitary. For example, Babcock *et al.* (1940), and Asdell *et al.* (1941), find that rats bred at an early age averaged 5.8 young at birth compared with 6.2 for those bred at normal age, and only 4.9 for those first bred late in life. Similarly, McKenzie (1928) found that sows bred for the first time late in life averaged only 5.6 young as compared with 8.4 young for those bred at the normal time.

Lactation

In many species of animals coincident lactation causes atrophy of the large follicles, and so for certain periods when lactation is at its height the animal is sterile. In the rabbit (Hammond and Marshall, 1925) this begins about the 9th day of lactation and continues until the end of suckling, while in the rat (Kuramitsu and Loeb, 1921) and mouse (Kirkham, 1916) the anoestrous condition exists for only a short period at the height of lactation. In the sheep suckling delays the onset of the breeding season (Roux, 1936; Hammond Jnr., 1944). In cases where the ova are shed and fertilised before the lactation atrophy of the follicles and ovary occurs, pregnancy is prevented by foetal atrophy (rabbit) or prolonged beyond the normal duration (rat, mouse) (*see* p. 700). In the sow ovulation does not normally occur until a few days after weaning, but if injections of F.S.H. are made during the suckling period, oestrus, ovulation and pregnancy can be obtained (Cole and Hughes, 1946; Zawadowsky (B.), 1945). In other species, such as the cow for example, there is no interference of the

normal ripening of the ova during lactation : but whether or not this is due to a different balance of the anterior pituitary hormones in such species is not yet known.

In other species again, as for example in women, there is considerable variation between individuals in this respect ; some menstruate and some do not during the suckling period. Long-continued lactation is believed to reduce the fecundity of women, who sometimes refrain from weaning their babies in the belief that by doing so they are less liable to become pregnant again. Haddon's (1908) observations upon Eastern Islanders of the Torres Straits show that, with these people also, prolonged nursing tends to reduce the size of the families and that a single lactation may be continued for three years.

In species in which this individual variation in reproductive activity during lactation exists, the level of nutrition may be the determining factor. In the islands of Shetland (*see* Goudie, 1891) the pony mares living under poor nutritional conditions rarely produce a foal more than once in two years when suckling their foals, whereas mares of the same breed living under better nutritional conditions in England will produce a foal every year. This breeding in alternate years when not suckling is frequently found in species in phosphate deficient areas (*see* p. 658). Rats which are not allowed to suckle their young and which are re-bred immediately after parturition produce many more young than those subjected to lactation (Babcock *et al.*, 1940, Asdell *et al.*, 1941).

Not only does suckling, removal of the anterior pituitary, under-nutrition, anoestrus, etc., cause atrophy of the follicles, but a certain amount of follicular degeneration is always going on in the ovary in relation to the different phases of the cycle. Various types of follicular atrophy exist : in the rabbit the percentage atresia among the young oöcytes is low while it is frequent among follicles of larger size (Pincus and Enzmann, 1937). The mature follicle does not persist indefinitely in the oestrous rabbit, but is rather short-lived, so that during oestrus numbers of follicles appear and disappear (Smelser, Walton and Whetham, 1934 ; Hill and White, 1933). In the rabbit, too, a special type of atrophy exists, in which the cavity becomes filled with blood (Hammond and Marshall, 1925, *see* Chapter 5).

Nutrition

Loeb (1917) who studied the effects of nutrition on the production of ripe follicles in guinea-pigs, found that under-feeding, if very pronounced, prevents maturation in all cases and causes atrophy before the follicles have reached medium size, and that this leads at least to temporary sterility ; the results are more marked in the ovaries of young animals, just as the general effect is greater. In extreme cases he states that "hypotypical" ovaries may be produced in which the connective tissue between the follicles is affected resulting in a union of follicles and a consequent polyovular condition. Parkes (1928a), too, found that deficiency of vitamin B in the ration caused the abrupt cessation of oestrus in rats in about 4 weeks. Such animals will, however, ovulate if injected with anterior pituitary (Marrian and Parkes, 1929). A low nutritive condition, especially if associated with exposure to wet and cold, as in the case of cattle wintered in the open air is often a cause of temporary barrenness (Wallace, 1907). In cobalt deficient areas the onset of oestrus and ovulation in sheep at the beginning of the breeding season

is often much delayed : this can be cured by the feeding of cobalt (Dunlop, 1944). In certain areas, such as on the South African veld, which is mineral deficient, the addition of phosphate in the form of bone meal increases the percentage of heifers which come on heat (Quinlan *et al.*, 1941) and the calving percentage from 56 to 87 (Du Toit and Bisschop, 1929) ; similar conditions have been found in America (Hart and Guilbert, 1928). Roux (1936) found in Merino sheep that reduction of the ration reduces the duration of the sexual season, particularly in the case of younger sheep. That the food supply does not alone regulate the time of the breeding season, however, is shown by the fact that sexual activity persists in spite of declining body weight and that the anoestrous period corresponds to the period during which the greatest increase in body weight occurs. Kelley (1937) also found that the plane of nutrition of the ewe had no association with the failure of oestrus to appear. An account of endocrine relationships in nutritional reproductive failure has been given by Guilbert (1942).

The effect of the food supply on the number of follicles which ripen at each oestrus is most marked in the sheep in which species the number of lambs born is closely related to the number of ova shed. Marshall (1903) found from an examination of Scottish sheep that the normal number of ova discharged at any single oestrous period is not appreciably in excess of the usual percentage of births at the lambing season ; this has been corroborated by Clark (1934). It would seem probable, therefore, that a scarcity of twin births at lambing is the direct consequence of an abnormally low number of ripe follicles in the ovary at tupping time. Aristotle (1910) commented on the increased fertility of sheep in a favourable environment and Darwin (1905) states that :

“ the amount of food affects the fertility of the same individual ; thus sheep which on mountains never produce more than one lamb at birth, when brought down to lowland pastures frequently bear twins. The difference apparently is not due to the cold of the higher land, for sheep and other domestic animals are said to be extremely prolific in Lapland.”

Heape (1899b), who investigated the fertility of sheep in England, found that the fertility of a flock depends greatly on its management and the quality and quantity of food supplied.

Precise records of the effect of “ flushing ” or artificially stimulating the ewes by means of special food at the approach of the tupping season were obtained by Marshall (1908) in an investigation undertaken in Scotland. Statistics showed that in the flocks treated in this way the percentage of lambs per 100 ewes was almost invariably in excess of the average percentage for flocks which received no special treatment ; in some cases the number of lambs in the flushed flocks was nearly 200 per cent. White and Roberts (1927) have also shown that, when Welsh ewes remain on the mountains before tupping they produce only 90 per cent., while those which have been kept in the lowlands where the food is better average 123 per cent. Marshall and Potts (1921) found, too, that by flushing so that the ewes gained 7 lb. per week during the mating time the percentage fertility was increased from 129 per cent. to 147 per cent. Similarly, Underwood and Shier (1941) found that ewes flushed so as to weigh 123 lb. as compared with the controls' weight of 98 lb. produced 114 per cent. lambs as compared with 94 per cent. for the controls. Clark (1934) has also shown that flushing leads to a higher

ovulation rate in sheep. Hart and Miller (1937) found that a low vitamin A intake did not reduce the number of lambs born although low protein and phosphorus did (Miller, Hart and Cole, 1942). In dry areas of Australia the percentage of lambs obtained follows the rainfall and so the nutrition available in different years (Stewart and Moir, 1943). Good young grass and rape are very efficient feeds for flushing sheep and it may possibly be that this effect is due to their content of gonadotrophic hormones (Friedman and Friedman, 1939). The presence of such substances in subterranean clover in W. Australia has, however, been suggested as a possible cause of large outbreaks of sterility and dystocia in sheep (Gorrie, 1946). Buffon (1802) among others remarked on the fact that domestic animals breed oftener and produce larger litters of young than wild animals belonging to the same species; and Darwin (1905) who made the same observation, attributed the increased fertility of the former to a long habituation to a regular and copious food supply without the labour of seeking it. "The wild rabbit is said to breed four times yearly and produce each time at the most six young; the tame rabbit breeds six or seven times yearly, producing each time from four to eleven young." While the number of corpora lutea found in the ovaries of wild rabbits, and so the number of ova shed, averages 5·7, in medium-sized domestic rabbits they average 10·3 (Hammond, 1921). King (1939), who followed the life processes of the wild rat (*Rattus norvegicus*) during 14 years in captivity found that whereas females of the first generation produced only an average of 3·5 litters those of the 19th generation averaged 10·2 litters, and that sterility and low fertility of females had disappeared by the 10th generation.

Darwin (1905) remarks that birds afford still better evidence of increased fertility resulting from domestication. Thus, in its natural state the female of *Gallus bankiva*, the wild representative of the common fowl, lays only from 6 to 10 eggs; the wild duck lays from 5 to 10 eggs as compared with 80 to 100 or more produced by the domestic duck in the course of the year. Since Darwin's time rapid improvement has been made: the record is now held by an Australian Runner duck which produced 303 eggs in a year, while in the British National Test in 1925 a Khaki Campbell duck laid 357 first grade eggs in the year.

On the other hand, it is well known that wild animals when removed from their natural conditions and brought into captivity often become partly or completely sterile. Darwin discusses this phenomenon at some length, and cites numerous cases from different groups of animals and birds (see Chapters 1 and 13).

The Indian elephant, cheetahs and various hawks, for example, seldom breed in captivity although kept in a perfectly healthy condition with exercise in their native countries. On the other hand, most members of the *Suidae* are known to breed in zoological gardens, while ruminants breed readily in climates widely different from their own. Carnivorous animals breed somewhat less freely in confinement and show considerable variation in different places. The *Canidae* tend to be more fertile than the *Felidae*, while members of the bear group breed less easily. Rodents as a general rule fail to breed after being brought into captivity, but there are several exceptions.

Among birds, members of the hawk group very seldom breed in captivity. The graminivorous birds show considerable variation, some, like the canary, breeding freely in aviaries (although it was some time before it became fully

fertile), while others like the finches only occasionally reproduce their kind when kept in confinement. Gallinaceous birds, on the other hand, show an unusual capacity to breed in captivity, and the same is the case with pigeons, ducks and geese. Certain kinds of gulls are known to breed readily when kept in open spaces in zoological gardens.

The causes for the failures among certain species of wild animals to breed in captivity are not yet completely understood. It may be that the changed nutrition has something to do with this failure to breed. Fattening domestic animals for shows, for example, is said to be a cause of sterility.

Marshall and Peel (1910) have described abnormal ovarian metabolism in fat cows and heifers, and Kirkham (1920) records sterility in mice which after four to six litters lay down extensive quantities of fat. Parkes and Drummond (1928) could not, however, affect fertility in rats by making them fat. In man obesity is known to be a cause of sterility (Cooper, 1918). It should be remarked, however, that over-fatness may be a result, as well as a cause, of infertility. It has been suggested (Heape, 1931) that in some cases as, for example, in the elephant in the wild state, the regular seasonal migrations for the purpose of breeding are connected with the seeking out of special food substances suitable for reproduction. Heape also attributes the periodic immigration of the lemming to variations in the nature of the food-supply leading to periodic increase in the rate of reproduction. Among wild birds, especially, migrations are closely linked with reproductive activity (Rowan, 1930).

An attempt to analyse the causes which govern the rate of reproduction in a wild species has been made by Baker and Ranson (1932a, b, 1933) on the field-mouse; they found that, while temperature and food supply had some effect, by far the most important factor concerned was the length of daylight. It seems probable that a large number of the cases of failure among wild animals to breed in captivity may be attributed to the lack of such exteroceptive factors (*see* Chapter 13). Monkeys and apes, which are particularly liable to be infertile in captivity, have in many cases responded to treatment by ultra-violet radiations, as, for example, in the colony of marmosets kept at the Lister Institute (Marshall, 1936) in which treatment resulted in a very great increase in the rate of multiplication (*see also* p. 662).

The Breeding Season

The rate of reproduction in many species is limited by the fact that breeding is confined to certain seasons of the year. In the domestic fowl, in which eggs are produced all the year round (instead of being limited to one season of the year as occurs in the wild jungle fowl), the rate of production is much greater at some seasons than at others. The main cause for the seasonal variation of egg production in the domestic fowl is probably the variation in hours of daylight at different times of year. Fronda (1928) and Whetham (1933) have shown that the seasonal curve of egg production shows a close correspondence to that of the hours of daylight in different latitudes (Fig. 21. 3), while Kennard and Chamberlain (1931) and Hofman-Bang and Holm (1932) among others have shown experimentally that the winter egg production of fowls is increased by addition of electric light after dark. The ovaries of starlings are activated in winter by the addition of a light

ration (Bissonnette, 1931), and this can be used to advance or prolong the egg-production period in pheasants (Bissonnette, 1937, Bissonnette and Csech, 1941). The light probably acts on the anterior pituitary gland to increase its gonadotrophic activity for, as Witschi and Keck (1935) have shown, injection of gonadotrophic hormones will cause the ova of sparrows to ripen in the non-breeding season (*see* Chapter 13).

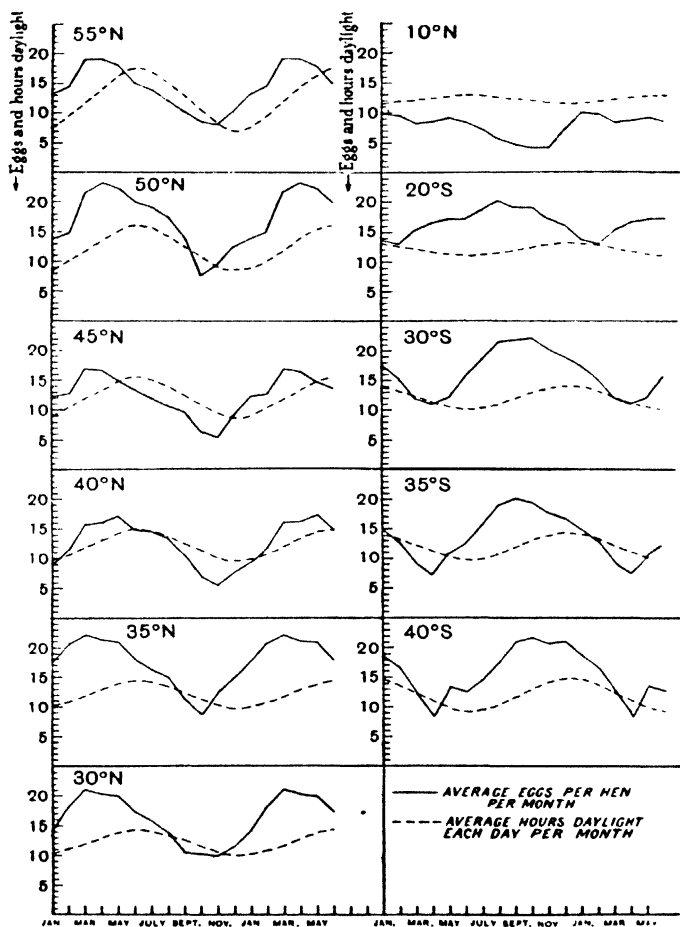


Fig. 21. 3—The relation between the hours of daylight and the seasonal egg production curve of fowls in different latitudes. (From Whetham, 1933.)

The breeding seasons in mammals appear also to be controlled in the same way (Baker and Ranson, 1932a). Just as plants can be classified into spring or autumn flowering according to whether the development of the flowering shoots are stimulated by lengthening or shortening daylight hours, so there exist in mammals species (ferret, horse) which only breed in the spring and summer and others

which only breed in the autumn and winter (sheep (Fig. 21. 4), deer). When such species are transferred across the equator they either immediately, or after a short period for accommodation, fit in their breeding seasons to their new daylight environment (Marshall, 1936, 1937; Bedford and Marshall, 1942). As with birds, the addition of an extra light ration will cause summer-breeding mammals to commence breeding during the dark winter months (Bissonnette and Bailey, 1936, for ferrets; Bissonnette and Csech, 1937, for raccoons, etc.), and in mares the onset of oestrus in spring can be accelerated by irradiation with strong artificial light, this being associated with the shedding of the winter coat (Burkhardt, 1947a). It is evident that both the duration and intensity of the illumination are important (Marshall and Bowden, 1934), and that, provided these are constant, the particular

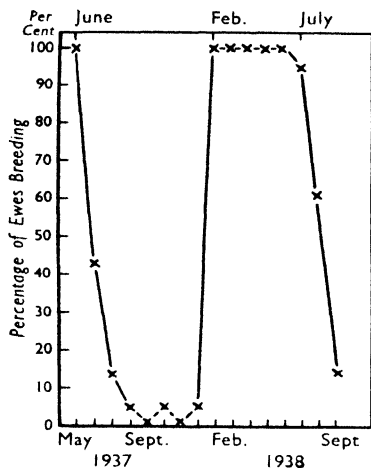


Fig. 21. 4—Curve showing the percentage frequency of occurrence of oestrus in Border Leicester Merino ewes in Australia. (From Kelley, 1939.)

part of the spectrum from which the rays come is immaterial, except that those coming from the ultra-violet end may have particularly lasting effects (Marshall and Bowden, 1936). Within the tropics, where the sun is overhead twice during the year, there are two periods of maximum ultra-violet light, and Baker (1937) suggests that this may be the reason for the fact that many species in the tropics have two breeding seasons within the year. Villegas (1928), for example, finds that in the Philippines sheep have two breeding seasons and not one each year, the peaks occurring in June and December. As one goes north from the equator in temperate latitudes there is a general tendency for the egg-laying seasons of birds of all kinds to start later and later at the rate of some 20 or 30 days per 10 degrees of latitude (Baker, 1938). Bedford and Marshall (1942), however, find that mammals inhabiting tropical areas when taken to temperate areas do not react to the seasonal influence of daylight duration, probably because they do not possess a developed mechanism of response to seasonal influences in their own countries. The mechanism of the action of light on the ripening of ova is thought to be by an effect on nerves in the region of the eye whereby the anterior pituitary is stimulated to increased activity. So far little experimental work has been published on the effects of shortening of daylight hours on the autumn-breeding animals in the way that Garner and Allard (1930) have done for the chrysanthemum, in which species there are critical lengths of daylight for different varieties. Kelley (1939), however, has shown that with sheep kept under the same light environment there are well-marked and constant differences between breeds and even strains in the time of onset and end of the breeding season. Bissonnette (1941) has shown that goats, a winter breeding species (Asdell, 1926; Phillips *et al.*, 1943), can be made to breed in the summer by giving an extra light ration in winter and then reducing it as summer approaches. This has also been done for sheep by Sykes and Cole (1944) and Yeates (1947); the latter finds that breeding begins

some 10 to 14 weeks after the change to decreasing length of day is made and that the onset is independent of the actual light ration at that time. Roux (1936), in South Africa, has shown that on constant feed conditions throughout the year, Merino ewes will fail to come into oestrus at certain times of the year (September to January); he found great individual and breed variation in the length of the breeding season, and considers that these are due to genetic causes. While the sexual season in the British mutton breeds is about 100–150 days in length in mature ewes, it is shorter in ewe lambs (Cole and Miller, 1935; McKenzie and Terrill, 1937; Hammond Jnr., 1944) and occurs in the northern hemispheres at the same time (September to January) as the non-breeding season in the southern hemisphere. Kelley and Shaw (1939, 1943) also find genetic differences in the length of the breeding season; Merinos have a relatively long breeding season, Dorset Horns slightly less, while Border Leicesters have a relatively restricted one. Hammond, Jnr. (1944), found that in Britain the breeding season extends from early October to late March and is fairly evenly spaced about the shortest day over a period where the time from sunrise to sunset is $11\frac{1}{2}$ hours or less. He found that within the breeding season the average number of lambs born per fertile service rises at first quite quickly to a peak for services in November and then declines steadily.

Owing to conditions affecting survival at birth, genetic control of reproduction has, under natural conditions, often become linked with daylight hours. Thus egg production in hens is linked with increasing hours of daylight and in sheep with decreasing hours, varying in degree according to the latitude of the origin of the breed (Hammond, 1947).

Since the reason for the non-breeding season is fundamentally the lowered output of follicle-stimulating hormone by the anterior pituitary, the obvious method of extending fertility into this time of the year would appear to be by injections of follicle-stimulating hormone; but the matter is not so simple as it would appear at first sight owing to species differences. It is comparatively easy to cause the follicles to ripen and ovulate (Hill and Parkes, 1930), but other conditions are necessary, such as the occurrence of oestrus and the correct timing of this in relation to ovulation (*see* p. 679), before fertility can be induced; this applies particularly to the sheep, in which the first ovulation in the breeding season normally occurs without any external symptoms of oestrus (Grant, 1934). Cole and Miller (1933) and Zawadowsky *et al.* (1935) found that injections of gonadotrophic hormones into anoestrous ewes caused ovulation, usually between 24 and 36 hours afterwards, but did not cause oestrus; they obtained some pregnancies by forced service or artificial insemination on the first day after injection. McKenzie and Terrill (1937) state, however, that in such cases the time of ovulation may vary from under 36 to over 72 hours after injection, and so it would appear to be necessary to inseminate such ewes on two or three successive days (*see* p. 680). Murphree, *et al.* (1944) who inseminated ewes on the day of F.S.H. injection and again on the following day found lack of fertility when injection was made in the luteal phase (3 days after the beginning of oestrus) and during anoestrus, but fertility when injections were made in the follicular phase of the cycle (12 days after the beginning of heat). They have also observed lack of fertility in rabbits treated with pituitary extracts when pseudo-pregnant and suspect that failure of fertility is inherent in the ova at certain times in the cycle. Cole and Miller (1935) found that ovulation in sheep could be induced during the non-breeding season by injection of

50 r.u. or more of follicle-stimulating hormone and that the ewes did not show any signs of oestrus. When a second injection followed the first after an interval of 17 days (the length of the oestrous cycle), however, both ovulation and oestrus were produced and some fertile matings resulted. This result has been confirmed by Parkes and Hammond (1940) and Hammond Jnr. (1945), who also found that oestrus occasionally occurred after the first injection if there happened to be a corpus luteum in the ovary at the time of injection, cases of single ovulations unaccompanied by heat occasionally occurring in the non-breeding season. The difficulty of obtaining oestrus as well as ovulation after one injection of F.S.H. in ewes has been experienced by many workers (Underwood and Shier, 1942; Quin and van der Wath, 1943; Frank *et al.*, 1945) and it may be that the presence of an existing corpus luteum in the ovary is necessary to retard ovulation of the ripening follicle and allow time for the symptoms of oestrus to appear, such as normally occurs at the onset of the breeding season in sheep. For example, Noot *et al.* (1946) found that out of 177 anoestrous ewes injected with 250-350 r.u. of P.M.S. only 22 came into oestrus after one injection, but 114 mated after a second injection 16 days after the initial injection; of these 5 and 65 respectively produced lambs.

Sterility due to prolonged anoestrus is a fairly common occurrence in the bitch; Scorgie (1939) finds that in most cases injection of 187 m.u. of P.M.S. together with 50 m.u. of P.U. causes oestrus in 2 to 6 days and that this is followed by conception if mating occurs. During deep anoestrus in the mare all follicles and visible ova seem to disappear from the ovary (Burkhardt, 1947b) and in this condition injection of F.S.H. has no effect. Some other internal secretion is probably involved in the initiation of oögenesis at the beginning of the breeding season. In this connection it is suggestive that Bullough and Gibbs (1941) found that in both the mouse and the starling the production of new oögonia is at its maximum during very limited periods of the reproductive cycle, the post-ovulation period.

In the species which breed throughout the year, such as the sow, opinion seems to be divided as to whether the litters born at certain times of year are larger than at other times. While Johansson (1929) finds that the largest litters are born in February and March, Krizenecky (1935a) and Yamane and Makita (1934) could find no influence of the time of year on litter size although the latter found that the number of services required to produce a litter increased during the warm months of the year. In the cow, which also breeds throughout the year, Cole and Rodolfo (1924) find that a higher proportion of twins is born from services during October to December than at other times of the year, and Quinlan and Roux (1936) find that restriction of sunlight or exercise appears to shorten rather than lengthen the period between calving and the first subsequent oestrus. In rats, King (1927) could find no difference in the size of litters born at different times of year. (For man, *see* under Birth Rate, p. 712.) In wild rabbits where the breeding season extends from January to June in North Wales, the onset of the breeding season is determined by the females, but the close is determined by the males (Brambell, 1944) (*see further* p. 676).

Breed Differences

Within a species, breed has an important effect in determining the number of ova shed at each oestrous period. With fowls, for example, improved strains of

Leghorns will lay from 200-300 eggs per year, as compared with the 80-100 eggs laid by the table breeds, such as the Indian Game, and the 20 eggs or so of the wild Jungle fowl. Similarly with sheep, Heape (1899a), Nichols (1924, 1926b) and others, have shown that there is a wide difference between the average fertility of different breeds. In the rabbit there is in general a direct relation between the size of the breed and the number of young produced. This is also in general true for the different breeds of dogs. This difference in fertility is due to the number of ova shed; for example, a strain of large rabbits weighing 3,500 gm. will shed on the average about 10 ova, whereas a small strain of rabbits weighing 1,500 gm. will only shed 5 (Hammond, 1934a, b). Pickard (1930) found that while the large Flemish rabbit averaged 9 per litter and the moderate sized Chinchilla 6, the small Polish averaged only just under 4. The wild rabbit, which is small, averages 5 ova shed (Brambell, 1944). Gregory (1932) also relates the fertility and number of ova shed in rabbits to the weight of the breed; the small Polish with litters of 3.2 sheds 3.97 eggs, while the large Flemish Giant with litters of 10.2 sheds 12.88 eggs. MacDowell, Allen and MacDowell (1929) found, too, that the number of corpora lutea in mice is related to their weight; in wild rats not only are pregnancies more frequent among heavier animals but the number of young per litter is larger (Perry, 1945). It is possible that here again the growth and gonadotrophic hormones of the anterior pituitary may be concerned (*see* p. 656). the large strains with the higher egg production being those with the greater anterior pituitary activity. There is, as Hatai (1913) has shown for the rat, a high correlation between the size of the pituitary gland and body weight (for breed differences in fertility, *see also* p. 708).

Ovarian Control

Disturbances of the internal mechanism of the ovary which controls the production of ripe ova are frequently the cause of sterility. Normally the production of ovulation is inhibited by the presence of an active corpus luteum in the ovaries (*see* Chapter 5), and it is not until the corpus luteum begins to atrophy, or, in the case of the cow, is squeezed out of the ovary by pressure between the fingers exerted per rectum (Hammond, 1927), that a new follicle begins to ripen. In sheep, too, the removal of the ovary containing the newly formed corpus luteum results in the onset of oestrus after 2-4 days (McKenzie and Terrill, 1937). It sometimes happens in the cow that the corpus luteum persists, as it does during pregnancy, and prevents the formation of ripe follicles. The cause of the persistence of the corpus luteum in the absence of pregnancy is not yet fully understood, but Zschokke (1898) believed that it was caused by, among other things, the feeding of rye and brewers' grains in large quantities; brewers' grains act as a stimulant to milk secretion and it may be that a pituitary mechanism is again involved. By squeezing out the persistent corpus luteum per rectum about 80 per cent. of such sterile cows can be cured (Frei and Stäheli, 1926). Even in cases where the corpus luteum does not persist but the cow keeps turning to service every three weeks, and when no visible sign of disease exists, the squeezing out of the corpus luteum about the 10-15th day of the cycle in a very large proportion of cases cures the sterility (Hammond, 1936a; Rumianzev, 1938). The reason for this is probably that the removal of the corpus luteum throws the balance

of ovarian secretions over to oestrogen and so intensifies the oestrous changes in the uterus and cervix (*see* below). Another condition which causes sterility frequently met with in cows and other animals is that of cyst formation from Graafian follicles (Hess, 1921) which, instead of rupturing at the normal time, continue to enlarge and lose their granulosa cells (Frei and Finik, 1926; Hammond, 1927). Selye (1946) has given an account of these and other types of cysts and tumours of the ovary in woman. Speaking generally, cystic ovaries are of two types, those with multiple small cysts and those with one or more large cysts: the former are usually associated with a nymphomaniac and the latter with an anaphrodisiac condition. In extreme cases (*see* Pearl and Surface, 1915; Calder, 1927) the cow with cystic ovaries assumes many of the male sexual characters, her voice becoming low and deep like that of the bull while the tail head rises like a cow about to calve (*see* Fig. 21. 5). In the cow these cysts may be ruptured per rectum or vaginam; if they are of long standing, however, they may re-form. Rupture of these newly formed cysts before the granulosa is lost leads, however, to the formation of a corpus luteum and so gives a period of rest during which the ovary can regain its normal condition (Marshall and Hammond, 1937; Day, 1942a). Cystic ovaries in mares can also be cured by this means (Day, 1939b). In cows with cystic ovaries Casida *et al.* (1944) obtained a high percentage of pregnancies (36 out of 53 treated) after one intravenous injection of unfractionated extract of sheep pituitary gland. How far this cystic condition of the ovary is due to interference with the normal ovarian-pituitary mechanism between follicle-stimulating and lutealising hormones (*see* Chapter 12) is still a matter for conjecture. Ovarian cysts, however, can be produced experimentally in ewes and cows by injecting large doses of P.M.S. (500 m.u. and especially up to 2,000 m.u.) where a corpus luteum is present in the ovaries (Zawadowsky *et al.*, 1935a, b; Folley and Malpress, 1944); this also occurs in sows where luteal cysts are formed which persist for a long time in the ovary (Chivatov, 1935).

The explanation of the varied results of gonadotrophic hormone injection in different species and the liability of certain species to particular causes of sterility probably lies in differences in the pattern of the oestrous cycle in different species (Asdell, 1946) or in the natural balances between the levels of F.S.H. and L.H. in the pituitaries and between the levels of oestrogen and progesterone from the ovaries (Hammond, 1946b). For example, while in the mare the anterior pituitaries are particularly rich in F.S.H., in the cow L.H. predominates, and resulting from this the follicular phase of the cycle is dominant in the mare and the luteal phase in the cow. When the activity of the anterior pituitary becomes reduced, as it does at the non-breeding time of year and for other causes, the mare is liable to failure to ovulate because the L.H. fades away before the F.S.H., while the cow fails to ripen follicles before failure to ovulate occurs, for injection with F.S.H. during anoestrus results in ovulation; the sheep behaves very much as the cow in this respect. The ripening follicle takes a long time to ovulate in the mare, which often results in long heat periods and sterility through death of the sperm before ovulation (*see* p. 682) and in some cases the follicle regresses without ovulation occurring at all (Andrews and McKenzie, 1941). If, when the follicle has reached a fairly mature stage in the mare 1,000–2,000 i.u. of P.U. is injected intravenously, ovulation will occur 24 to 48 hours afterwards (Day, 1939a, 1940); this method is now being used to increase fertility in horse-breeding practice.

In anoestrous sheep, where frequently a large follicle exists in the ovary, ovulation can be caused by injection of stilboestrol (Hammond, Jnr., 1945) : this probably acts by evoking ovulation-producing activity on the part of the animal's own pituitary.

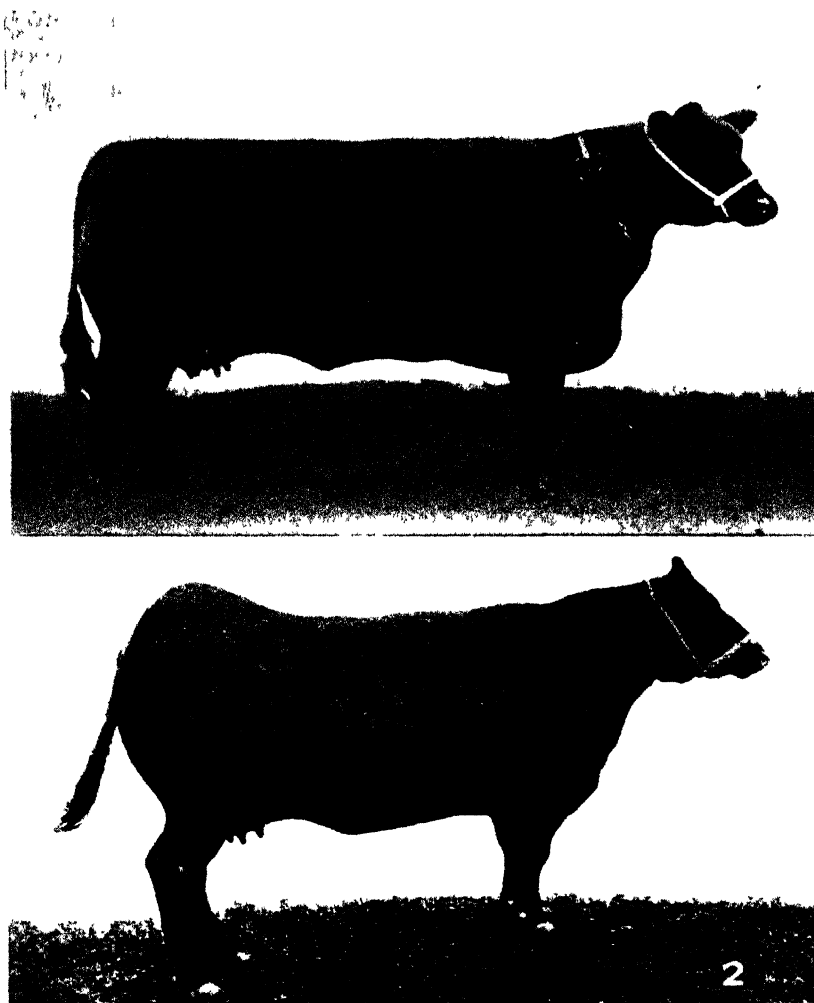


Fig 21 5 Aberdeen Angus cow (1) with normal ovaries and (2) with cystic ovaries
(From Calder, 1927)

Experimentally, a condition of anoestrus in the ovaries can be produced by the implantation under the skin of tablets of the synthetic oestrogen, stilboestrol (Hammond Jnr. and Day, 1944), which probably acts by reducing the gonadotrophic activity of the anterior pituitary, for when such cows are injected with large doses of P.M.S., follicles may ripen and ovulation occur (Hammond Jnr.

and Day, 1944). When the anoestrous condition is very deep, however (i.e. with very atrophic ovaries), even very large injections of gonadotrophins fail to grow a follicle and this is so under some conditions of natural anoestrus in the cow and at all times in the mare (Day, 1940 ; Burkhardt, 1947b). It may be noted, too, that stilboestrol is capable of inhibiting the induction of ovulation in the sheep by P.M.S. if given before, or too soon after, administration of F.S.H. (Hammond Jnr., 1945) ; the stilboestrol probably causes the anterior pituitary to shed its L.H. into the blood before the P.M.S. has caused follicles to reach ovulation size.

The naturally occurring anoestrous condition of the ovaries in young cows is much more frequent in the north of Britain than in the south. There is some evidence that as one proceeds south a milder form of the condition occurs, which consists of the ripening of the follicle and ovulation without the symptoms of oestrus occurring—the so-called “ silent heats.” These “ silent heats ” also occur naturally in the ewe at the onset of the breeding season, where regularly oestrus does not occur until the second ovulation after the onset of the breeding season (Grant, 1934). As an explanation of these “ silent heats ” it would appear that the balance of L.H. is greater than F.S.H. in both the sheep and the cow and that under lowered anterior pituitary activity the F.S.H. fades out at a greater rate than the L.H. As a result of this the balance between oestrogens and progesterin is such that the symptoms of oestrus do not occur ; this balance on the progesterin side is naturally found in pregnancy, and has been experimentally produced with suppression of the symptoms of oestrus by tablet implantation in ferrets (Marshall and Hammond Jnr., 1945). In cows with “ silent heats,” too, the squeezing out of the corpus luteum with the hand per rectum, by reducing the excess progesterone, leads to the resumption of oestrus (Folley and Malpress, 1941). The opposite condition—that of the appearance of oestrus with a large follicle that does not ovulate, but regresses—is of frequent occurrence in the mare, a species in which, as pointed out above, the balance is on the F.S.H.—oestrogen side. Experimentally such a condition is also often produced in the cow shortly after long standing, implanted stilboestrol tablets have been removed, i.e. after saturation of the body with oestrogens. Under such conditions follicular cysts are frequently found in the ovaries but become absorbed and normal cycles resumed as the anterior pituitary regains its full activity (Hammond Jnr. and Day, 1944). Under reduced anterior pituitary gonadotrophic activity, such as in adolescence in chimpanzees (Young and Yerkes, 1943), ovulation is believed to occur and the relative sterility of this period is thought to be due to a deficiency of luteal function.

Other Causes

Sterility and reduced fertility due to lack of proper ova formation is frequently to be found in crosses between varieties and species. Darwin (1872) discussed this at some length, and, among his seven main conclusions on this question, found that there are all degrees of cross infertility ; that many cases are known of species which can be crossed with ease, but yield hybrids excessively sterile ; and conversely, some which can be crossed with great difficulty, but produce fairly fertile hybrids ; and that the degree of sterility of first crosses and of hybrids runs to a certain extent parallel with the general or systematic affinity of the forms which are united. Wallace (1897) has cited several cases in which it has been

shown that hybrids between distinct species are fertile *inter se*. Such cases are the hybrids between the domestic and Chinese geese, and the various hybrids between the different species of the genus *Canis*. A case of a fertile hybrid between a lion and a jaguar has also been recorded. The various members of the family *Bovidae* are known to hybridise and to produce fertile offspring even though the parents belong to what are ordinarily regarded as different genera. The cow (*Bos taurus*) gives fertile male and female hybrids when crossed with the zebu (*Bos indicus*). With the yak (*Bibos grunnicus*), the guar (*Bibos gaurus*), the gayal (*Bibos frontalis*) and the bison (*Bison americanus*), the cow has fertile female hybrids, but sterile males (see Morgan, 1913; Hamer, 1925; Babcock and Clausen, 1927). The hybrids between the cow and *Bison bonasus* are similar (Iwanow, 1911a, b, 1913a; Iwanow and Philiptschenko, 1916). Crosses between the yak and zebu also give fertile female offspring, but the male hybrids of both F₁ and backcross to the parents are sterile, due to lack of spermatozoa formation in the testes (Zawadowsky, 1926, 1931). These and other observations show that sterility among hybrids between closely allied species, although usual, is very far from being universal (Suchetet, 1897; Dewar and Finn, 1909). Investigation of the male sterility in cattle-buffalo hybrids has shown (Deakin *et al.*, 1941) that the sterility is due to the fact that the hybrids inherit the closed scrotum of the buffalo and so the temperature is too high for spermatogenesis in cattle and the first cross (see p. 680).

The discovery of the part the chromosome and gene play in inheritance now gives a general explanation of such cases of sterility, although the details of the process whereby the ova and sperm fail to mature properly have only been worked out in a few cases. It should be mentioned, however, that the chances of obtaining a species hybrid are not indicated by the chromosome numbers. Much more advance has been made in plants in this respect owing to the greater ease with which they can be controlled. In some cases the fertility of a hybrid may be produced by doubling its chromosome number (Karpechenko, 1937). Experimentally, haploids and polyploids can be produced by various means such as treatment with chemicals, low temperature, narcotics, etc. (Imp. Bur. Plant Genetics, 1936) and particularly by colchicine (Blakeslee and Avery, 1937; Sears, 1939); pollination with a genetically distinct species capable of inducing parthenocarpy but not fertilisation can also be used for inducing haploid parthenogenesis. Pincus (1939) has attempted something of this sort with mammals by treatment of the tubal egg of the rabbit and subsequently transplanting it to the tubes of a pseudopregnant doe, and obtaining parthenogenetic development. Such methods may possibly in the future be applied to those animal hybrids in which reproduction is not possible owing to cytological abnormalities.

Before the ovum can be fertilised maturation and formation of the polar body have to occur. The first maturation division occurs in the rabbit in follicular ova only after copulation; that is, it is initiated by the action of L.H. on the ovary. This may account for some of the difficulties found in obtaining *in vitro* fertilisation of human ova obtained directly from follicles before normal ovulation, as has been found by Rock (1945) and although explantation alone may lead promptly to the first maturation division (Pincus and Enzmann, 1935) it may be that this is not altogether normal. In monkeys, like most mammals, the first maturation division takes place in the follicle only just preceding its rupture (Hartman and Corner, 1941).

Incomplete development of the ovaries and ova may be brought about also by other means. The "freemartin" (a heifer co-twin to a bull calf) has, as first described by Hunter (1792a), incompletely developed ovaries and so is sterile. Lillie (1917) has suggested that this is due to influence of the internal secretions of the bull co-twin, brought about by fusion of the two foetal circulations during pregnancy; Bissonnette (1930) has shown that one male may affect two females in this way (*see* Chapter 22). Not all heifers born twin to a bull are freemartins however; statistics show that a normal female may be expected about once in twelve cases of twinning involving both sexes (Swett *et al.*, 1940). Freemartins can now be experimentally produced as a result of the multiple pregnancies obtained after injection of F.S.H. (Hammond Jnr. and Bhattacharya, 1944).

Pseudo-hermaphrodites, having outward characters of the female with an incompletely developed female tract, and cases of abnormal development of the male organs, are common in goats and are found rarely in some other species. In goats there is some evidence for a heredity basis for these abnormalities, a recessive gene (in the case of goats linked with the polled character) being responsible for the abnormality in development (Asdell, 1936, 1944; Eaton, 1943). This explanation has also been suggested by Baker (1929) for the high frequency of the ceremonial intersex pigs found in the New Hebrides (*see also* Witschi, 1939).

Incompletely developed ovaries have also been reported in pigs by Deakin (1932). Moreover, the application of X-rays to the ovary in appropriate doses causes destruction of the ova and follicles without interference with its other functions (Bouin, Ancel and Villemin, 1906; Bergonié and Trabondeau, 1907; Specht, 1907; Parkes, 1926). In birds where the completed egg is the product of the whole reproductive tract as well as the ovary, various abnormalities may be found such as double yolked and dwarf eggs: an account of these has been given by Asmundson (1931).

III. THE NUMBER OF OVA FERTILISED

Various factors affect the number of ova fertilised. Not only is the number and vitality of the sperm produced by the male important, but all those factors which make for easy ascent and prolongation of the life of the sperm in the female tract come into play. Not the least of these are the time relations that exist between mating and ovulation, for both the sperm and ovum have a very limited independent life and, unless time relations are properly synchronised, the chances of fertility are considerably reduced. The development of new methods for the collection and preservation of the sperm outside the body have, in recent years, led to considerable extension in the use of artificial insemination in breeding, and here again the time relations between insemination and ovulation are of the greatest importance for full fertility.

Because only one sperm is required to fertilise an ovum, and because normally very large numbers of spermatozoa exist in the ejaculate, it was formerly considered that the male was relatively unimportant as a determiner of fertility, and that with the male it was a question of either full fertility or sterility. Recent work, however, has shown that this is not the case and that the production of large numbers of vigorous sperm is essential for maximum fertility. Walton (1927), for example,

by successively diluting semen and inseminating doe rabbits, found that the size of the litter varied directly with the density of the sperm suspension, and that with a higher degree of dilution complete sterility resulted, even though the suspension contained many thousands of spermatozoa. Chang (1946a) found, too, that while 475,000 sperms were sufficient to fertilise all eggs (9) of the rabbit, a reduction to 92,000 resulted in a 57 per cent. fertilisation only. If, however, the number of ova to be fertilised (24-73) was increased by superovulation with gonadotrophic injections no more sperms were required. The reason why so many sperm are required to effect fertilisation is not only that of their dispersion through the genital tract of the female, but also probably the effect of the hyaluronidase contained in them, for this disperses the cells surrounding the ovum and allows of the penetration of the ovum by a spermatozoa. Deficiency of this enzyme in some semen may possibly account for unexplained cases of sterility (McLean and Rowlands, 1942), but few observations on this have yet been made. Leonard and Kurzrok (1945) report 5 successful cases of insemination in man after the addition of bull hyaluronidase to the semen which had previously failed to produce pregnancies.

Sterility without the loss of other sexual functions may be produced by the operation of vasectomy in males. Males so treated are used as "teasers" in modern stockbreeding methods and are of use in many physiological experiments on fertility and the oestrous cycle. The operation may be used on man, for eugenic purposes, to prevent propagation of the feeble-minded, etc.

Sperm Production

There are many causes for lack or deficiency of spermatozoa production by males. As mentioned above (p. 669) in many hybrids there may be complete lack of spermatozoa production due to defects in the chromosome and gene pattern of the hybrid. Similar chromosome aberrations have been suggested (Doncaster, 1914) to account for the sex-linked character which is the cause of the well-known rarity and sterility of the tortoise-shell male cat. A genetic basis to explain abnormal spermatozoa in two species of doves and their hybrids has been suggested by Shrigley (1940).

Much sterility and lowered fertility is caused by defective sperm production in man (Moench and Holt, 1931, 1932; Williams, 1943; Harvey and Jackson, 1945; Hotchkiss, 1945), stallions (Anderson, 1922; Walton and Fair, 1928), bulls (Hammond, 1921; Williams and Savage, 1927; Savage *et al.*, 1927; Lagerlöf, 1934; Sciuchetti, 1938), rams (McKenzie and Phillips, 1934; Constock and Brady, 1937), boars (Rodolfo, 1934; Phillips, 1935; McKenzie *et al.*, 1938), rabbits (Hammond and Marshall, 1925) and other species. Surveys of rams in New Zealand have shown that some 13 per cent. are sterile or of very low fertility (Webster, 1939a). Nicol (1934) finds in the guinea-pig that the "prenatal mortality" is 29 per cent., which is chiefly due to lack of fertilisation of the ova shed. In such cases the sperm may be deficient in number, abnormal in their form, reduced in vitality, or a combination of these conditions may exist. Methods for detecting these conditions are described by many of the workers quoted above. The technique employed consists, after collection by the artificial vagina method (*see* p. 691), of volume determination, sperm counts to determine density, staining

methods to determine abnormalities (*see* Fig. 21. 6) and determinations of the rate of gaseous exchange under standard conditions (Walton and Edwards, 1938) to determine vitality. When the sperm are inactive, and the alkaline secretions of the accessory glands predominate in the ejaculate, the pH of the semen becomes more alkaline (7.0 or over) : this can be used as a field test for fertility in rams



Fig. 21. 6—Progressive degenerative changes of the spermatozoa taking place in the semen of a bull from a period of normal fertility to one of sterility. a. normal sperm ; b. with a large number of free protoplasmic globules ; c. curled tails ; d. heads and tails separated. (From Lagerlöf, 1934.)

(Webster, 1938, 1939b). Another quick method which is widely used is that of determining the rate at which the sample decolorises methylene blue under anaerobic conditions (Salisbury *et al.*, 1943 ; Beck and Salisbury, 1943 ; Larsen and Sørensen, 1944 ; Perry *et al.*, 1945). In a good sample of bull semen the blue colour should disappear in $3\frac{1}{2}$ to 6 minutes, while a bad sample will require some 9 minutes or more. This method is also used in Denmark as a quick method for determining the number of active sperm in samples of bull semen, reduction times of 3, 6, 9 and 12 minutes corresponding to 700,000, 630,000, 570,000 and

500,000 sperms per mm.³ respectively (Larsen and Sørensen, 1944). For quick estimation of the numbers of sperm in a sample of semen a sediment method has been used by Gunn (1936) and an opacity method described by Burbank (1935) and by Kyaw (1944). Gunn (1936) found in aged rams that the semen was often contaminated with urine and that this shortens the life of the sperm. The various types of abnormalities found are described by the workers quoted above, particularly Lagerlöf (1934). McKenzie and Phillips (1934) state that rams showing more than 140 abnormal spermatozoa per thousand are probably of reduced fertility, provided the semen sample has not been taken immediately following a long rest period. Laing (1945a) found that on the basis of density, total number of sperms, motility and change in pH, he could divide bulls into high and low fertility groups requiring 1.0 to 3.5 and 3.5 to ∞ services respectively per conception obtained. He could, however, find no relationship between the percentage of abnormal sperm and the service rate.

Various causes for this defective sperm production have been suggested. Much of the literature on the factors affecting semen production has been given by Anderson (1945). Marshall and Hammond (1937) conclude that under-feeding and over-use in the young animal, and under-use and overfeeding to the degree of fatness in the old animal, reduce fertility. Parkes and Drummond (1928) could not, however, produce lack of spermatogenesis in rats by fattening them although they caused disinclination to copulate. In old age, about 14 years and over, the fertility percentage of travelling stallions is reduced (Sanders, 1926). Lauprecht (1932) finds in horses that while the incidence of twins increases with the age of the mother it decreases with the age of the father. Voronoff (1920) claims that males in decrepit old age may be rejuvenated and regain fertility by testis grafts: an account of these experiments is given by Marshall *et al.* (1928). Phillips and Andrews (1936) found that low nutrition retards the production of spermatozoa in the boar, while Cunningham and Hopkirk (1935) obtained sterility in male rats by feeding excessively high protein diets. Among other causes are vitamin B deficiency in pigeons (Marrian and Parkes, 1928) and rats (Parkes and Drummond, 1925), vitamin A (Parkes and Drummond, 1926) and E (Mason, 1925; Kennedy, 1926b; Korenchevsky, 1933; Cabell and Ellis, 1940) deficiency in rats. Accounts of these and other dietary deficiencies causing sterility are given by Kennedy (1926a) and Mason (1939). Lack of exercise and fresh green food is suggested as a cause of sterility in bulls (Miller and Graves, 1932). Severe vitamin A deficiency in young cattle has been shown to cause a cystic condition in the pituitary gland which suggests a possible mode of action on fertility (Madsen *et al.*, 1942). Under ordinary farm conditions the feeding of sprouted oats (Miller and Graves, 1932), and the feeding of animal proteins (milk, blood meal) as distinct from vegetable proteins (Smirnov-Ougryumov, 1937) to bulls with deficient sperm production have been found to increase their fertility. It is stated that the addition of hens' eggs to a normal ration has a good effect in keeping up the sperm content of semen in the case of stallions (Zwaenepoel and Royer, 1935) and of rams which are mating at the rate of 7 times daily during the breeding season (Lissov, Stoyanovskaya and Sharoyko, 1937); high protein feeding also increases sperm production in boars (Hudiakov, 1938). Danish experiments (Larsen and Sørensen, 1944) also confirm the good effects of a high protein ration on sperm production. It is said also to increase the viability of the

sperm. On the other hand, feeding on greenstuff increases desire and the volume of semen, but does not increase viability of the sperm (Laptev, 1940).

Cases have been described where the sperm deficiency in the semen was due to infections or blocking of the tubules in the epididymis (Corner, 1907); this is not infrequent in goats (Savage, 1929; Podhradsky, 1930) and has also been reported in rams (Gunn, 1942) and bulls (Anderson, 1938). Inbreeding, not as such, but because of the defective recessive characters it brings to light, has been mentioned by Hammond and Marshall (1925) as a cause of sterility in males.

It is well known that rigs or cryptorchids with the testes in the abdomen are sterile (Crew, 1922a; Nordby, 1928). There is some evidence that cryptorchidism is an inherited character (pigs, Nordby, 1933; sheep, Glembotsky and Moiseev, 1935, 1936). Injection of gonadotrophic hormone will cause descent of the testes in immature monkeys (Engle, 1930), but there is yet no evidence that it has any effect on natural cryptorchids. Small differences in temperature have a great effect on the production and life of the spermatozoa in mammals. Crew (1922b) first suggested that the cause for sterility in abdominal testes was the higher temperature that exists in the abdomen than in the scrotum. Experimentally the placing of the testes in the abdomen causes atrophy of the tubules (Moore, 1926a; Cunningham, 1927; Asdell and Salisbury, 1941a); this degeneration is produced quickly, in 10 days in rats (Leonard and Hamilton, 1937) and spermatogenesis ceases within 24 hours in the rabbit (Asdell and Salisbury, 1941b). After replacement in the scrotum such testes in the guinea-pig do not, however, regain their power of producing spermatozoa until after 90 days (Moore, 1926b). Young (1927) showed that water at 46° C. run over the scrotum of the guinea-pig caused germ cell degeneration, the effect of which on reproductive capacity reached a peak between the 44th and 75th day; later recovery of reproductive function occurred. Phillips and McKenzie (1934) showed that defective sperm and sterility could be produced by raising the temperature of the scrotum in the ram; in rams allowed three matings every third day, they found that it took from 4 to 13 days for abnormal sperm to appear in the ejaculate after treatment. They suggest that a heavy fleece and high "condition" frequently results in lowered fertility of rams by raising the temperature of the scrotum and that such animals recover their normal fertility if relieved of their fleece and fat. This is a frequent cause of low fertility in Merino rams, which have much wool on the scrotum, when kept under hot climatic conditions (above 90° F.) such as exist in parts of Australia, recovery not being complete until some two months after unfavourable conditions have ceased to operate (Gunn, Sanders and Granger, 1942). Phillips *et al.* (1943) also conclude that warm summer temperature may be an important factor in reducing sperm production and fertility in rams. In man, elevation of body temperature in conjunction with environmental temperature results in a marked drop in spermatozoa production which becomes apparent after about 3 weeks and may last for a period of 50 days thereafter (MacLeod and Hotchkiss, 1941). It is well-known, too, that some generalised diseases and feverish conditions of the body are often associated with temporary sterility and that this often occurs in rams after arsenical dips (Gunn, 1942; Gunn, Sanders and Granger, 1942). Conditions which give rise to greatly reduced body metabolism, such as thyroidectomy, or administration of thiouracil (Bogart and Mayer, 1946) have also been shown to cause decreased

sperm production (Krizenecky, 1932 ; Smelser, 1934 ; Zalesky and Wells, 1937). On the other hand, sperm production in normal rats is reduced by the administration of thyroxin (Smelser, 1939). Japp (1933) was able to hasten the onset of spermatogenic activity of drakes in the spring by thyroid feeding, while Berliner and Warbritton (1937) and Bogart and Mayer (1946) state that administration of thyroxin will prevent the normal summer decline in sperm production in rams (Green, 1940). Recently, promising results have been obtained by feeding iodinated casein to bulls with low sperm production (Turner, 1945 ; Reineke, 1946). The treatment of males which show defective sperm production under natural, as distinct from vitamin or mineral deficient diets, is, however, still in the experimental stage.

While removal of the anterior pituitary causes cessation of sperm production (Hill and Parkes, 1932b ; Allanson *et al.*, 1935 ; Liu and Noble, 1939), the results of injections of gonadotrophic hormones on sperm production are conflicting, and in practice, so far as increasing fertility goes, disappointing. For instance, Moore (1936) obtained no results from injections on sperm production in the rat, while Witschi and Keck (1935) obtained sperm formation in the testes of the sparrow in the non-breeding season by injection of follicle-stimulating hormone, but none from injections of lutealising hormone. In ground squirrels injection of A.P. and P.M.S. caused precocious sperm formation (Wells and Moore, 1936) and sperm production after hypophysectomy (Wells and Overholser, 1938). Wells (1938) finds that the gonadotrophic potency of the anterior pituitary of the male is very low during the summer non-breeding season when no sperm are being produced, but that this seasonal reduction is prevented by constant low temperature. Chang (1942) found that in rams sperm production was temporarily increased (for about 5 days) 8 days after implantation of stilboestrol tablets. Evidence that special substances circulating in the blood stream form the basis of the level of sperm production in the testes in the breeding season in the adult

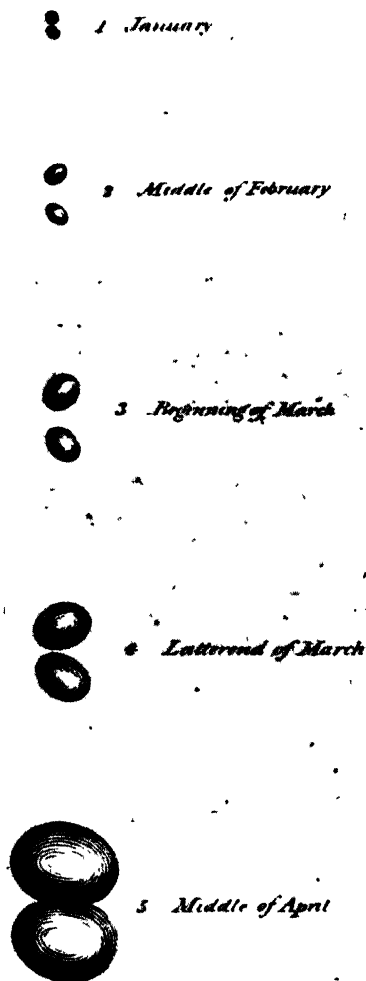


Fig. 21.7 - Changes in the relative size of the testes in the sparrow during the onset of the breeding season (From Hunter, 1792.)

is still, however, far from complete. While removal of one testis leads to compensatory hypertrophy in the remaining testis in cocks and drakes (Domm and Juhn, 1927 ; Benoit, 1931) the evidence is not clear in mammals. Smelser (1933) found increased sperm production from the remaining testis in rats, but his results have been criticised by Edwards (1940), who, on careful quantitative investigation, found no effect at all on the sperm production of the remaining testis in rabbits when one was removed. It has been found that, in the fowl, the cocks of high egg-laying strains produce more semen than do those of low egg-producing strains (Jones and Lamoreux, 1942).

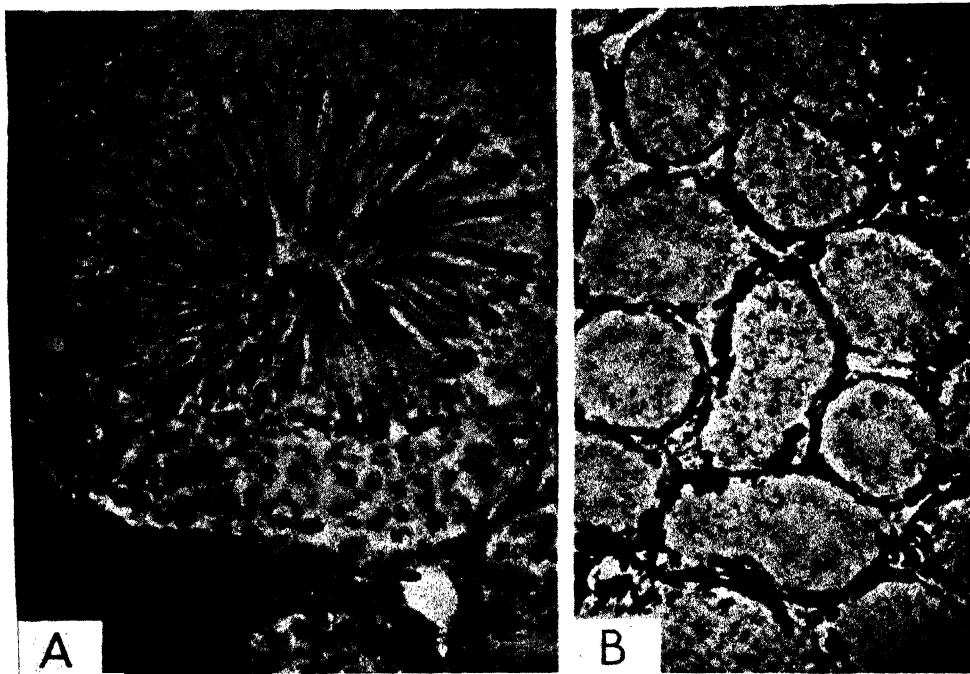


Fig. 21. 8—Sections through the testes of starlings in winter.
A. Following a period of extra light ration after dusk. B. Normal, not lighted.
(From originals by Bissonnette and Wadlund, 1932.)

The rate of sperm production by the testes does, however, vary greatly at different seasons in the year in most birds and in some mammals. Hunter (1792b) first described (Fig. 21. 7) the enormous increase in the size of the testes of the common sparrow that takes place from January to April in England. These changes can be induced by changes in the light ration (Fig. 21. 8), in the same way that the production of ova by the ovary can be influenced (Bissonnette, 1931, starling ; 1935, ferret ; 1939, blue jay ; Riley, 1937, sparrow). By injections of pregnant women's urine hormone following light treatment, fertility and pregnancy have been produced during the non-breeding winter months in ferrets (Allanson *et al.*, 1934). Bottomley *et al.* (1940) have also reported the restoration of fertility in 3 bulls treated with P.U.

In those species which have a winter breeding season, such as the sheep, the desire and power of mating may exist in the male during the non-breeding season, but there is a decrease in spermatogenic activity and an increase in the numbers of abnormal sperm. This result could be produced in the winter breeding season by keeping the rams in rooms at high temperatures (McKenzie and Berliner, 1937); the Hampshire breed showed much less seasonal difference in this respect than did the Shropshire.

A more special cause of sterility in man is one which operates in the case of workers with radium or the Röntgen rays. Albers-Schönberg (1903), Regaud and Dubreuil (1907), Brown and Osgood (1905) and others have shown that the X-ray causes degeneration of the cells lining the seminal canals without diminution of sexual potency. Hertwig (1938) finds that in mice the greatest effects of sub-sterilising doses of X-ray on the fertile matings and litter size of such treated males are produced after a period of 8 days or more after irradiation, that is, the effect is greater on the spermatozoa in process of formation than on the mature sperm (Eker, 1937). The effects of X-ray treatment are shown not only on destruction of the sperm themselves, but also in producing chromosome changes which cause the production of lethal mutations.

In order that a high proportion of the ova shall be fertilised, it is not only necessary that the male shall produce large numbers of normal sperm, but also that the time at which the mating is made in relation to the time of ovulation offers the best condition for fertilisation. In many species this is provided for by the short space of time during which the female will accept the male, this time coinciding with the optimum conditions for fertilisation. In other species, however, such as in man and the monkey, where mating is allowed at any period of the oestrous cycle, and, to a lesser extent, the horse, in which the duration of oestrus is long, a knowledge of the conditions which determine the optimum time for mating are required for the control of fertility. A knowledge of these conditions will also be necessary to appreciate the causes for sterility under abnormal conditions in those species which have a short oestrous period.

Like the animal itself, the spermatozoon goes through a life cycle (*see* Chapter 7). After its formation in the testicular epithelium it passes fairly quickly through the tubules, where it is usually in an immature state and for the most part has not yet achieved the state of motility; consequently, spermatozoa obtained directly from the testis are not usually capable of fertilising ova (Young, 1929a, 1931; Munro, 1938b). After passing quickly through the tubules of the upper epididymis, the spermatozoa accumulate in the lower epididymis where they come to maturity. The sperm becomes motile and the protoplasmic drop (derived from the epithelium of the testes), which is at first situated near the head, gradually passes down towards the tail, gets smaller and then disappears as the sperm becomes mature (*see* Chapter 7). Selivanova (1937) believes that the loss of the drop is not due so much to maturity as to the influence of secretions from the accessory glands, and that changes in the nature of these lead to the retention of the drop by the sperm on ejaculation under certain circumstances. Young and Simeone (1930) and Young (1931), however, have demonstrated the necessity for the maturation of the sperms in the epididymis; only 33 per cent. of guinea-pigs inseminated with the immature sperm from the testicular end of the epididymis become pregnant as compared with 68 per cent. with mature sperm from the

proximal end : when, however, the sperm was confined in the epididymis for longer (25 days) periods by ligature of the vas, the conditions were reversed and only 25 per cent. became pregnant from sperm from the distal end, as compared with 49 per cent. from the proximal end. Kirillov and Morosov (1937) and others find that in bulls the second ejaculation, as regards all sperm indices, is better than the first one, which may be very variable, especially where there is a long interval after the previous mating.

The sperm maintain their motility in the lower epididymis up to 60 days and their power of fertilising ova up to 38 days in the rabbit (Hammond and Asdell, 1926) ; when, however, the epididymis is anchored in the abdomen these times are reduced to 14 and 8 days respectively (Asdell and Salisbury, 1941b). In the guinea-pig the motility of the sperm is retained for 65 days (Benoit, 1925 ; Moore, 1928) and fertility for 30 days (Young, 1929b) and 42 and 21 days respectively in the rat (White, 1933). In the bull, progressive movement of the sperm continues for 37 days and an oscillating movement for about 60 days (Kirillov and Morosov, 1936). The life of the sperm in mammals is sustained in the epididymis by secretions from the lining epithelium, for after the male hormones affecting these secretions are withdrawn by removing the testes the sperm of the guinea-pig remains motile only about 20 to 23 days (Benoit, 1925 ; Moore, 1928). In birds, however, sperm maintain their motility in the vas for 27 days irrespective of the presence or absence of functional testicular tissue (Munro, 1938a). It is significant that the period of motility of the sperm in the epididymis extends beyond the period of their power of fertilising ova. It is probable that only in their prime of life are sperm capable of fertilising ova and that as this power wanes they may maintain motility for some little time before disintegration sets in. In the semen of males which have not mated for some time, many such disintegrated sperm—with the heads separated from the tails—may be seen in the distal portion of the vas deferens (Simeone and Young, 1931) ; these occur in enormous numbers under conditions where the vas deferens or epididymis becomes occluded (Tamura and Crew, 1926 ; Moore and Quick, 1924). These facts lead to a consideration of what is the optimum time between copulations necessary for obtaining the highest degree of fertility in the male. From measurements of the volume of the ejaculate, the density and appearance of the sperm and the time the spermatozoa would remain motile after collection, McKenzie *et al.* (1938) concluded that in normal boars the maximum production of effective sperm occurred when the interval between copulations was at intervals of 48 hours or more ; there was some indication that in boars of low spermatogenic activity it was somewhat longer. Thus at intervals of 12 hours between collections, the duration of the motility of the sperm was only $2\frac{1}{2}$ days whereas at intervals of 72 hours the sperm remained motile for $8\frac{1}{2}$ days. For other species there is at present very little exact evidence of what the optimum interval between matings is, although Lloyd-Jones and Hays (1918) found in the rabbit that the fertility of the buck, both in the number of pregnancies and in the size of the litters, was considerably reduced by a succession of matings at short intervals. Macirone and Walton (1938) have shown that the number of sperm in the ejaculate of rabbits falls from 73 to 13 millions for the 1st to 6th ejaculate made within a day.

In normal rams the ejaculate may be from 0.6 to 1.5 c.c. in volume and contain from 300 to 450 million sperm, having a concentration of 3 to 5 million sperm

per mm.³ Individuals vary greatly in the rate at which sperm exhaustion occurs in successive copulations over a short period of time. Thus McKenzie and Berliner (1937) found that while some rams even on the 19th copulation during one day produced 0.66 c.c. semen containing 1 billion sperm with a density of 2 million sperm per mm.³, others produced only 0.1 c.c. ejaculate containing only 60 million sperm on the 5th copulation and in some instances would refuse even to mate. Similarly in man, Belding (1934) found great individual differences in the rate at which the sperm become exhausted in successive ejaculations; in one case where the number of sperm averaged well over 500 million with intervals of 10 to 14 days the number with successive 12 hour intervals dropped to 233, 238 and 38 millions. Such "exhaustion tests" form one of the best methods of measuring male fertility in low fertility males (Walton and Edwards, 1938). Chang (1945) from experiments with normal, good fertility rams concluded that in general the content of the epididymis does not alter very considerably with different rates of ejaculation, but that the number of spermatozoa is kept relatively constant either by increased absorption during periods of sexual repose and infrequent ejaculation, or by increased stimulation of spermatogenesis due to ejaculation during periods of sexual activity.

Time Relations between Mating and Ovulation

In most cases the time of mating is strictly limited to the short period of oestrus which occurs before ovulation. By experimentally altering the normal time relations between insemination and ovulation it has been determined that in most mammals the separate life of sperm and ova in the female tract is of very limited duration. These facts form the explanation of many cases of low fertility and sterility. The length of time that spermatozoa can retain their fertilising power and motility in the female tract varies considerably in different types of animals; in general these differences appear to be related to temperature conditions. In the queen-bee one copulation is sufficient for a lifetime production of fertile ova. In fish, which are poikilothermal, van Oordt (1928) found that fertilised female specimens of *Xiphophorus helleri* kept isolated from males for more than 10 months gave birth to several subsequent litters. In many birds where the testes are abdominal and so normally subject to high temperatures the life of the sperm in the female is also comparatively long. In the turkey a whole clutch of 20 to 30 eggs laid at about daily intervals is fertilised as the result of one treading, while in the domestic fowl fertile eggs may be laid up to about 10 to 20 days after a mating, although the percentage fertility begins to fall rapidly after about the 5th to 7th day (Gilbert, 1904; Laurie, 1912; Elford, 1915; Crew, 1926; Chlebaroff, 1930, etc.). Iwanow (1924) thought that the whole clutch of growing oöcytes was fertilised within the hen's ovary; Walton and Whetham (1933) could not, however, support his conclusions, while Hartman (1937) has pointed out that experiments by Warren and Kilpatrick (1929) definitely show that this is not so, for when the cocks were changed eggs fertilised by the last cock were laid as early as the second day after the change had been made (*see further* p. 683). Chlebaroff (1930) found that in the fowl the number of eggs fertilised from one copulation depends both on the rate at which the hens are laying and on the sexual vigour of the cock: in his experiments it was up to 8 eggs. Riddle and Behre

(1921) found that in ring-doves the spermatozoa retained their fertilising power for 8 days, while in the duck, Chappelier (1914) found absolute sterility after the 7-11th day from mating. In hibernating mammals such as in many bats where copulation takes place in the autumn, but ovulation does not occur until the spring, the spermatozoa live through the winter in the uterus and fertilise the ova when they are shed in the spring (Courrier, 1927; Redenz, 1929; *see* Chapter 8). In all the other types of mammals, however, with scrotal testes, where the temperature is normally below that of the abdomen, the length of time the spermatozoa remain capable of fertilising ova after their deposition in the female tract is a matter of hours or a few days only. This time was found to be about 30 hours for the rabbit (Hammond and Asdell, 1926); in cases where the sperms had remained in the female tract for more than 24 hours small litters were frequently produced. Similar results have been obtained in the guinea-pig by Soderwall and Young (1940): the limit of time during which fertilising capacity was retained by spermatozoa in the female tract was 22 hours, and after 17 hours litter size and the percentage of females pregnant were reduced. Corresponding times for the rat are 14 hours and 10 hours respectively (Soderwall and Blandau, 1941). Lewis (1911a, b) states that in the pig in most cases the sperm cells are dead within 24 hours of service and only exceptionally are they found alive longer than 48 hours. Feebly motile sperm have been found in the uterus and tubes of the guinea-pig up to 41 hours after copulation and up to 17 hours in the rat (Yochem, 1929). In the bitch, Whitney (1927) found only dead sperm at 3 days and slightly motile ones at 2½ days. Quinlan, Mare and Roux (1932a, b) believe that in the ewe the cervix acts as a reservoir for sperm from which there is a continuous issue up the tract; although they find that by 48 hours after mating all the sperm in the genital tract are immotile. Kelley (1937) finds that in the female tract the fertilising power of sperms from even the most fertile rams was lost at about 34 hours after copulation; Lopyrin (1938) gives this time as 36-40 hours, while Anderson (1941) finds that ram sperm remain fertile in the genital tract of the ewe for 22½ hours. In the cow the sperm remain capable of fertilisation for about 28 hours (Laing, 1945b), ovulation occurring about 14 hours after the end of oestrus. In the horse in which ovulation usually occurs about 24-48 hours before the end of the oestrus, matings or inseminations are only occasionally fertile when made 7 days before the end of oestrus (Hammond, 1938; Day, 1942b) which would seem to indicate a life at the most of about 5-6 days. In the stallion, however, the scrotum is held more closely to the abdominal wall than in the ram and bull.

One of the probable causes for this variation between species is the differences that exist between the temperatures of the epididymis and the female tract. In birds where the testis is abdominal and the spermatozoa can live at high temperatures their life in the female tract is much prolonged as compared with mammals in which the spermatozoa are stored in the epididymis at temperatures below that of the abdominal temperature (*see* p. 674). In the bats which hibernate during winter the body temperature falls during this period. When the isolated epididymis of the rabbit is placed in the abdomen the sperm only remain fertile for 4 days instead of the 40 days which they remain fertile when the epididymis is in the scrotum (Knaus, 1932). Popa (1931) and Popa and Marza (1931) who studied the modifications of the spermatozoa in the female tract found that they were

quickly destroyed and put the survival at about 24 hours. Yochem (1929) found that in a non-oestrous uterus the spermatozoa are destroyed even more quickly.

Owing to the short life of the spermatozoa in the female tract of most mammals, there is an optimum time for mating to take place before ovulation occurs. The scope of this time-variation naturally varies with the species and is of more importance in species with long oestrous periods (such as the horse) than in those with short ones, such as the zebu which has an average oestrus of just over one hour (Anderson, 1936). That there is an optimum time for mating can be demonstrated in the rabbit for which it has been shown (*see* Fig. 21. 9) that the number of ova fertilised decreases gradually from full fertility in matings made at the normal time (10 hours before ovulation) to small litters, of 1 or 2 only, at 20 hours before ovulation (Hammond and Asdell, 1926). From post-mortems of inseminated does it was found that after the sperms had been in the female tract

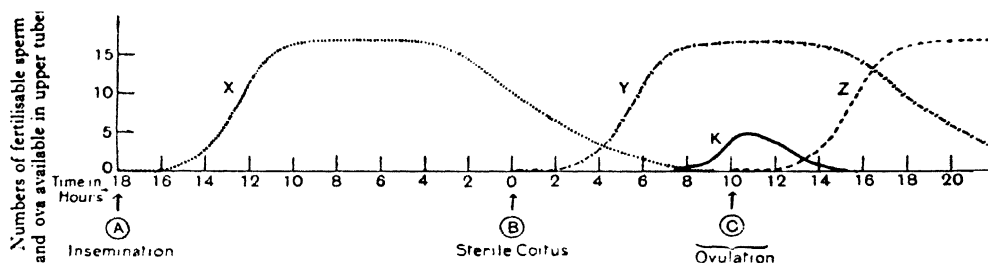


Fig. 21. 9—Diagram illustrating the possible causes of small litters due to length of vitality of sperm and ova in the rabbit. K. Curve of available (upper tube) ova, due to their short life. X, Y and Z. Curves of available (upper tube) sperm, due to the time taken to reach the upper tubes and to the short life in the female tract.

X. From insemination (A) at 18 hours before sterile coitus (B) and 28 hours before the mean time of ovulation—small litter produced. Y. From a normal fertile coitus at (B) 10 hours before the mean time of ovulation—normal large litter produced. Z. From a fertile coitus at (C) made 10 hours after a sterile coitus at (B)—small litter produced. (From Hammond and Asdell, 1926.)

24 hours before ovulation 41 per cent. of the ova shed failed to be fertilised, after 26 hours 50 per cent., and after 28–34 hours 100 per cent. Similarly, in the case of those animals which have a long heat period (1 to 37 days for the mare, Andrews and McKenzie, 1941) and which produce only one young at birth such as in the mare, the percentage of matings which prove fertile decreases from about 67 per cent. in those made 1 day before ovulation, to 50 per cent. at 4 days, and 29 per cent. at 5 days before ovulation, while all matings made 7 days or more before ovulation prove sterile (Hammond, 1938a). This is probably the reason why the fertility of the travelling stallion, which is mated with mares at any time in the heat period, is lower (53 per cent.) than that of the Thoroughbred (66 per cent.) which stands at stud and tries mares every two days during the heat period, and still lower than that of the mountain pony stallion (95 per cent.) which runs with the mares all the time. This is not so much a matter of breed but of mating at the right time, for Anderson (1922) finds that when a band of brood mares is turned on to a pasture with a stallion 90 per cent. will become pregnant. Schtschjekin (1930) found that mating the mares twice during the oestrus on the 1st and 5th days gives better results than mating on the first day only.

Constantinescu and Mauch (1936) obtained similar results ; mating mares once only during the oestrus resulted in 40 per cent. fertility, whereas mating twice, on the 2nd and 5th days, raised the fertility to 57 per cent. They also found the chances of fertility were much reduced when mares had long oestrous periods of over 5 days. The optimum time for service of the mare is, according to Shkurgin (1939), the 4th day before the end of the oestrus, while Samodelkin (1939) puts it at 12-24 hours before ovulation at a time when, as can be determined by rectal palpation, the whole ovary begins to soften as a preparation for ovulation. Shchekin (1936) points out the uselessness of serving mares during long heat periods as it causes an unnecessary waste of the stallion's energy. By injecting 1,000 m.u. of P.U. into mares, Mirskaya and Kedrov (1938), Day (1939b, 1940) and Hamilton

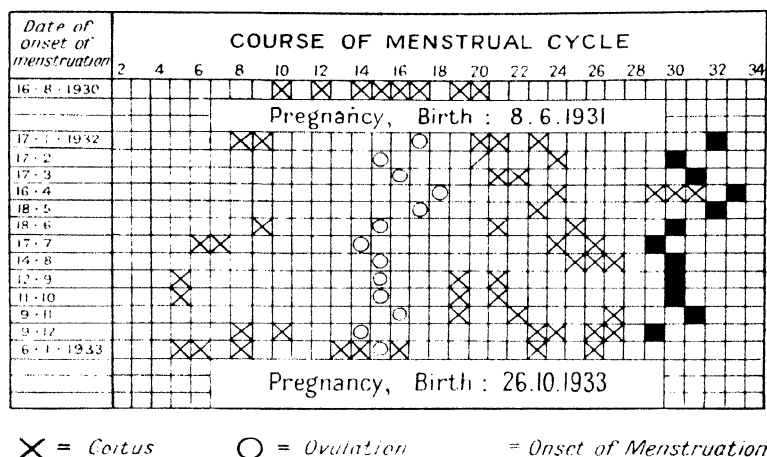


Fig. 21. 10.—Chart of the sexual life of a woman, showing how abstinence from coitus for several days before and a few days after ovulation results in sterility, whereas coitus just before and just after ovulation results in pregnancy. (From Knaus, 1934.)

and Day (1945) reduced the duration of oestrus by causing ovulation 30-34 hours after injection and so diminished the number of inseminations or services necessary. The bitch has a long heat period of some 9-14 days (Schmaltz, 1921) and it is a common practice among dog-breeders to have their bitches lined twice during the heat period at intervals of two days in order to ensure that sufficient sperm shall be present when ovulation occurs. The vixen has a heat period of about 4 days and as the percentage of pregnancies is higher for matings on the 1st and 2nd than on the 3rd and 4th days of heat (Gunn, 1945) it would appear that ovulation frequently occurs before the end of heat. In monkeys the chances of fertility from a mating rise from a low level just before and after menstruation to a peak at about 12 days after the beginning of the menstrual flow, ovulation occurring about the 13-14th day (Hartman, 1932b, 1939a), and then drop again to sterility. In woman, too (Fig. 21. 10), much the same condition exists (Knaus, 1934 ; Ogino, 1930, 1932). By Jewish law mating is allowed after the 9th day from the beginning of the menstrual flow, the most fertile period of the cycle, and this race is notably a fertile one.

As males vary greatly in the numbers of sperm they produce (*see* p. 673), the time factor in mating becomes more important as the level of sperm production decreases. Differences in male fertility therefore become most marked in those species in which heat is of long duration or the time over which mating is allowed is extensive. Some stallions regularly give a smaller percentage fertility than others (Sanders, 1926 ; Nielsen, 1928 ; Speelman *et al.*, 1944) ; for example, Marshall and Crosland (1918) found that different stallions in one district varied from 27 per cent. to 75 per cent. in their fertility. Some difference does, however, occur in male fertility when mating takes place under natural conditions. For example, the percentage fertility for bulls on range may vary from 45 to 94 (Baker and Queensbury, 1944). Matings of a pure-bred recessive-coloured female with males of two different breeds within the same oestrous period can be used to verify the importance of the time of mating on fertility. Thus Crew (1926) found that, if after the removal of a cock from a pen of hens a second cock is introduced, the influence of the first sire is removed by the 7th to 10th day, the exact time differing in different cases. Similar double matings with time intervals have also been used to determine the optimum time for mating in the rabbit. Such double matings in quick succession, using a female with recessive characters, have been used to measure the comparative fertility (sperm swarm) of two males in the same way that Mendelian ratios measure segregation in the gametes of a cross-bred male (rabbits, Hammond, 1934b ; poultry, Bonnier and Trulsson, 1938).

Another factor which determines the optimum time for mating is that of the length of time the ovum remains capable of fertilisation after ovulation. In the domestic fowl, in which the egg takes about 24 hours to pass down the reproductive tract, it has been found that no egg laid within 24 hours after the time of mating is fertile (Coste, 1847 ; Gilbert, 1905 ; Chlebaroff, 1930), that is, the egg must be fertilised soon after it is shed if it is to be fertilised at all. Dunn (1927) from a number of tests concluded that the time elapsing between mating and the laying of the first fertile egg averaged less than 48 hours and had a minimum of about 21 hours. In the rabbit (an animal which only ovulates after copulation) mating normally occurs 10 hours before ovulation. If matings are made first with a sterile (vasectomised) male and later at a certain interval of time with a fertile male, the exact time that the ovum remains capable of fertilisation can be determined, for it is known that in the rabbit the spermatozoa take some 4 hours to reach the tubes. The results of such an experiment (*see* Fig. 21. 11) show that

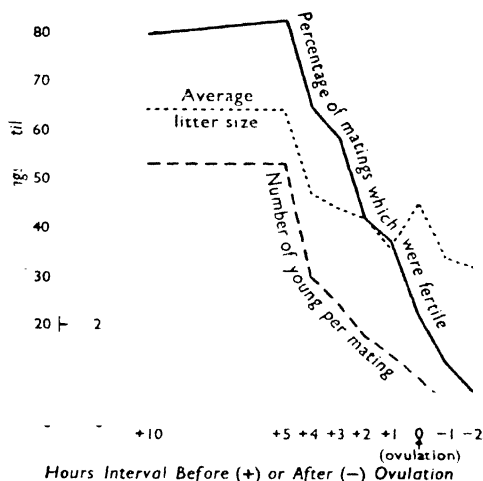


Fig. 21. 11.—Chart showing the fertility of matings made at different intervals of time before and after ovulation in rabbits. The time of ovulation was regulated by a previous mating with a vasectomised buck. Matings made more than two hours after ovulation were all sterile. (From Hammond, 1934b.)

matings made more than 2 hours after ovulation are completely sterile (Hammond, 1934b). The ova thus remain capable of fertilisation for some 6 hours after ovulation. This is about the length of time the ova remain in the ovulation plug at the top of the Fallopian tubes (Pincus, 1930, 1936) and it would appear that as they leave this and pass down the tubes fertilisation is prevented either by their movement, by the layer of albumen deposited around them, or by their senility. That they still remain alive after they have ceased to be capable of fertilisation has been shown by Pincus (1930), who has been able to obtain parthenogenetic cell division in them in tissue culture. That such eggs are truly parthenogenetic is suggested by the more advanced stages of parthenogenetic ova which Loeb (1932) has described in ovarian eggs in the guinea-pig. Incidentally, it will be seen from Fig. 21. 11 that small litters begin to be produced when the mating is made

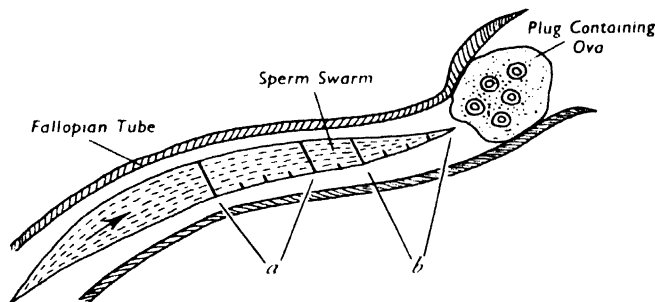


Fig. 21. 12—Diagram illustrating how the chances of the ova becoming fertilised are reduced as the interval of time between mating and ovulation is reduced in rabbits. The position of the sperm swarm is that which would obtain at about 4 hours after mating, and the marks on the sperm swarm denote hours which it would take to move upwards. As the plug exists for about 4 hours (in fertile matings where the sperm penetrate it) or 7 hours (where no sperms are present) sperms will accumulate for this length of time against the plug before it breaks down and the ova become incapable of fertilisation. (a) The amount of the sperm swarm which in a period of 4 hours would accumulate against the plug as a result of a mating made at the normal time—10 hours before ovulation. (b) The amount of the sperm swarm which in a period of 4 hours would accumulate against the plug as a result of a mating made 4 hours before ovulation. (From Hammond, 1934b.)

3–4 hours before the time when ovulation occurs; this is probably because under these conditions only the first one or two spermatozoa reach the top of the Fallopian tubes before the ova move out of the ovulation plug and so become unfertilisable (see Fig. 21. 12). The rate at which the ovulation plug in the tube liquefies and liberates the eggs is hastened by the presence of spermatozoa which contain a mucolytic enzyme. This enzyme also causes follicle cell dispersion from the ovum (Yamane, 1935; Pincus and Enzmann, 1936). In the ferret (Hammond and Walton, 1934) the ova remain capable of fertilisation for a longer period—some 30 hours—while, in the guinea-pig, the extreme limit of ovum viability is 26 hours (Young and Blandau, 1936). In the pig, Lewis (1911a, b) observed that the ovum seems soon to lose its vitality, for no results followed mating at a later date than one day after the period of heat had passed. Lowered fertility also follows services after the 30th hour subsequent to the onset of oestrus in the sheep (Quinlan, Maré and Roux, 1932a) although the sperm take only 6 hours to reach the upper end of the tubes after coitus. Ovulation in the sheep occurs at from 24 to 36 hours (McKenzie *et al.*, 1933; Schott and Phillips, 1941), or

according to one authority 30 to 32 hours (Lopyrin, 1938), or, in Merinos up to 40 hours, after the beginning of oestrus (Quinlan and Maré, 1931). Green and Winters (1935) found that in the ewe the sperm take 5 hours to reach the infundibulum and that the life of the ovum is probably less than 24 hours. Kelley (1937) has shown that the sperm travel up the reproductive tract of the ewe at the rate of 1.26 mm. per minute and reach the upper parts of the tubes in 5 hours, although Schott and Phillips (1941) put this at 4 cm. and 20 minutes respectively. Kelley finds that ovulation takes place just after or in the latter stages of oestrus. Schott and Phillips (1941) state that ewes of the Corridale and Dorset breeds tend to remain on heat after ovulation. Although the sperm reach the tubes in 5-6 hours their greatest concentration there is not until about 12 hours after mating in the ewe (Warbritton *et al.*, 1937) so that mating 12 hours before ovulation would seem to be the most desirable time in the ewe. In the mare, in which ovulation occurs about 24 hours or slightly more, and according to Mirskaya and Salzman (1935) occasionally up to 3 days, before the end of the oestrus, fertility is low (20 per cent.) for services made during the last 24 hours of oestrus (Hammond, 1938a, b; Day, 1942b). Neumann and Zalzman (1937) find that the best time to serve a mare is 10-20 hours before ovulation, while Day (1940) obtained good fertility from inseminating mares 12 hours before ovulation as determined by rectal palpation, but could not obtain fertility at all when inseminations were made 2 hours or more after ovulation. In man, Bryce and Teacher (1908) consider that the ovum is only fertilisable for 48 hours and similar conclusions have been arrived at by Triepel (1915) and Grosser (1924). Hartman (1932b) found from cases of isolated coitus in the monkey that the great bulk of conceptions occur from coitus between the 12th and 15th days of the cycle, and that except for one which occurred on the 19th day no conceptions occurred from matings made outside the 10th to 17th days of the cycle. Knaus (1934) arrives at much the same conclusion for man and states that the period during which conception can take place consists of the ovulation period (12th day for a 26 day cycle to 16th day for a 30 day cycle) plus 3 days before it and one day after it (*see* Fig. 27. 10). Allen *et al.* (1930) who recovered ova from the tubes estimate the time of ovulation in woman as between the 12th and 14th day of the cycle, which is much the same as that found for ovulation in the monkey (Hartman, 1944). The "safe period" which was suggested long ago for those of the Roman Catholic faith (Latz, 1933) coincides closely with these findings of modern science; the "safe period" comprises the last week or so of the cycle.

As with the animal itself and with the spermatozoon, the ovum goes through a life cycle, and cannot be fertilised until it is properly mature. The process of the ripening of the ovum as distinct from that of the follicle in which it is produced consists first in enlargement, and then in throwing off of a polar body. The production of this first polar body is normally dependent upon the pituitary hormones (Pincus and Enzmann, 1935; Pincus, 1936). As has been pointed out above, in most species the timing of oestrus and ovulation under natural conditions is such that the best chances are given for fertilisation to occur. Conditions, however, do arise in which this is not always so. Grant (1934) has shown that at the beginning of the breeding season in sheep ovulation may occur without oestrus. McKenzie *et al.* (1933) have observed in sheep that ovulation occurred in 6 per cent. of cases without the ewe showing desire for mating, while in 8 per

cent. of cases ovulation did not occur at oestrus. Roux (1936) has also reported "silent heats" in ewes, in which ovulation occurs without signs of oestrus both in the breeding season and in the anoestrus (*see* p. 668). In the ewe, Zawadowsky *et al.* (1935b) have shown that injections of gonadotrophic substances during the luteal phase of the cycle cause ovulation without the appearance of oestrus. In the cow, however, injection of either prolan or phenol during the luteal phase causes both ovulation and oestrus (Zawadowsky *et al.*, 1935a); the time relations between the two are upset, however, so that while ovulation occurs 48–60 hours after injection oestrus does not occur until 60–96 hours after injection. The cycle (and so implantation) is not governed by the corpora lutea so formed and this probably explains why pregnancy was not obtained (except in one case) even after artificial insemination just before ovulation (*see* p. 651).

Other Factors

Many abnormal conditions of the female reproductive tract may hinder the ascent of the sperm to the tubes and so may lead to reduced fertility or sterility. While in most species (cattle, sheep) ejaculation takes place into the upper end of the vagina (for sheep, Quinlan, Maré and Roux, 1932a), in a few, such as in the rat (Hartman and Ball, 1930) and horse (Hammond, 1938a; Hammond and Wodzicki, 1941) the semen is ejaculated through the cervix into the uterus. In the horse during oestrus it is no uncommon occurrence to find the cervix so constricted (at which time it should have been relaxed) that ejaculation takes place into the vagina and so the chances of fertility are greatly reduced (Hutschenreiter, 1915); mechanical dilation or artificial insemination is suggested as a remedy. In some species (those in which coitus is prolonged) it has been suggested that the semen is sucked through the cervix by rhythmic contractions, but this has been disputed (*see* Walton, 1930b; Dickinson, 1933); an account of experiments on this point has been given by Hartman (1932a, 1939a). It seems possible that in those species (ferret, dog, etc.) in which coitus is prolonged (Asdell, 1946) the penis may act as a plug in much the same way as the copulatory plug in rodents, to hold the sperm in the upper vagina for a sufficient length of time to ensure its entrance into the cervix. At one time it was thought that the filiform appendage on the ram's penis was necessary for fertility so as to introduce the semen into the uterus, but Iwanow (1929) has shown that this is not necessarily so, and that amputation of the appendage need not decrease fertility. Gunn (1936) observed that during ejaculation the appendage rotates and scatters the ejaculum, distributing the sperm round the cervix. In species in which ejaculation normally occurs into the vagina the ascent of the sperm may be hindered by metritis and cervicitis—a common cause of sterility in the dairy cow, according to Albrechtsen (1920) and Quinlan (1929). Sterility in dairy cattle has in recent years been increasing considerably in almost all countries (Edwards, 1933). The general opinion is that much of it is associated with infections of the reproductive tract. Sterility, as distinct from abortion, is frequently found as an aftermath of infection with the organism of contagious abortion; and trichomonas infection, a true venereal disease, which causes both early abortion and sterility (Emmerson, 1932; Riedmüller, 1933) has become widely spread in recent years. Granular vaginitis (McFadyean, 1909) has also been cited as a causal agent. Trichomonas infection

is frequently associated with persistent corpora lutea. As the trichomonads colonise in the uterus only while an active corpus luteum is present (Laing and Day, 1947) this disease may be treated either by squeezing out the corpus luteum or by injections of stilboestrol which throw the balance over to the oestrogenic side. Dimock and Edwards (1928) found that approximately one third of barren mares suffer from cervicitis and metritis due to a streptococcus. Constantinescu (1925) also observed pathological changes in the uterus in just under half the sterile mares examined. By suturing the superior commissure of the vulva, and so excluding air from the vagina such infections may be cleared up and fertility regained by recreating an anaerobic condition in the cervix and uterus (*see* p. 703). In some species, such as in the rabbit (Lush, 1925; Hammond, 1930), cow (Rowson, 1942) and in man (Müller, 1885), it is in the Fallopian tubes that inflammations and adhesions are most liable to cause obstruction to the ascent of the sperm; in man, gonorrhoeal infections are one of the primary causes for this condition (Duncan, 1884; Kelly, 1908). In sheep, sterility from causes of pathological origin is slight and constitutes only about 1–2 per cent. (Polovtseva *et al.*, 1937). In many species arrested development of some part of the female tract may occur and so the ascent of spermatozoa is prevented. Fincher and Williams (1926) have described such cases in the cow associated with inbreeding. A cause for sterility frequently cited by medical and veterinary practitioners (Fleming, 1878) is that of acidity of the vagina and cervix for which irrigation with bicarbonate of soda is recommended. Within the limits of acidity found in the vagina and the time of its action on the sperm under ordinary conditions there would seem to be some doubt as to whether such acidity could destroy the vitality of the sperm (Smith and Asdell, 1941, and Asdell *et al.*, 1942, for the cow). Kato (1936) finds that horse spermatozoa agglutinate at between pH 6.4 and 5.0 and believes that this is a normal process after copulation. Grodzinski and Marchlewski (1938) find cocks' spermatozoa are agglutinated by cock serum, but believe that this agglutination has a serological basis for this property is destroyed by heating the serum. Chang (1946b) finds that there is a spermicidal factor in fresh human, bovine, rabbit, guinea-pig and rat sera which is not species specific and is thermolabile. It is destroyed by sodium citrate and has other characteristics of complement. The effect of acid on the consistency of the mucus of the cervix and upper vagina may possibly prevent the ascent of the sperm, although Green and Winters (1935) find that sperm reach the infundibulum of the ewe out of heat almost as quickly as when the ewe is on heat and the sperm will pass through the uterus of a ewe 17 days pregnant. Lopyrin (1938) concludes, however, that during the cycle (outside the heat period) the sperm cannot ascend the female tract in the sheep, because pregnancy could not be obtained from ovulations caused by gonadotrophic hormone injections at this time. These results are, however, capable of another explanation (*see* p. 686). Sjövall (1938), however, found in guinea-pigs that, while the spermatozoa easily pass the cervix by their own motility during oestrus, their passage is practically excluded in the non-oestrus phase and in castrates; their passage is, however, re-initiated by injecting castrates with oestrin. It is conceivable that circumstances which give rise to incomplete liquefaction of the mucus of the cervix at oestrus lower fertility (Hammond, 1939). In woman at the time of ovulation, about mid-period, and for 3 or 4 days preceding, there is an outpouring of clear glairy mucus from the cervical canal and in this the sperm are

much more active than in the more tenacious mucus of other periods of the menstrual cycle (Williams and Simmons, 1942). Many substances inserted into the vagina and used as contraceptives will destroy the vitality of the sperm, but the power of the normal body acids in this direction, even in high concentration, is very small as compared with contraceptive substances such as toluquinone, hexylresorcinol (Baker, 1935) or phenyl mercuric acetate (Baker, Ranson and Tynen, 1939). In most species the semen contains an alkaline reserve, which varies, however, in different individuals. The average ejaculation from man measures 3.9 c.c., contains 461 million sperms, and requires 3.1 c.c. of N/100 HCl to neutralise it (Baker, Ranson and Tynen, 1937). There is little evidence as to the effects of drugs upon egg or sperm production, but innumerable substances have been recommended as a cure for impotence. Cantharides and various other drugs are said to produce sexual excitement, but this result is probably simply due to the increased flow of blood to the generative organs which these substances induce (Bloch, 1908). Yohimbine probably acts in much the same way (Daels, 1908; Cramer and Marshall, 1908). Phosphorus, caffeine and strychnine have also been used for the same purpose. Injection of 1,000 rat units (or about double the dose necessary for the female) of gonadotrophic hormone into impotent rams will produce sexual libido and normal breeding, although it will not affect the sperm content of the semen (Hart and Cole, 1936). Such impotence often occurs early in the breeding season in rams. In bulls, too, injections of P.M.S. and prolan will cure impotence (Soldatenkoff and Shneerson, 1935). Such results show promise for the future control of impotence, while other methods, such as that of electrical stimulation of the ejaculatory centre in the spinal cord in rams (Gunn, 1936) and the rectal massage of the vasa deferentia in bulls (Miller and Evans, 1934) are now available for the collection of semen from impotent animals for insemination purposes (*see* p. 692). As a consequence of the widespread use of artificial insemination in cattle breeding, attention has been drawn in recent years to various forms of "acquired impotence" and "psychological sterility" in bulls. This has been the subject of recent research in the U.S.S.R. and already some general principles have emerged indicating the cause and method of treatment. According to Milovanov and Smirnov-Ugrjumov (1944) the conditioned sex reflexes leading to ejaculation may be inhibited by: (a) strange surroundings developing the "What is that?" reflex which inhibits the sex reflex; (b) by not strengthening the conditioned sex reflex by immediate service, as when bulls are kept tied up in cowsheds with cows in oestrus; (c) by pain experienced at the time of service, by defective technique of sperm collection; or (d) by too frequent services under exactly identical conditions. Among the conditions which they have found effective in breaking down these conditions are: arranging for service in a quiet place (a); keeping bulls and cows apart until the time for service (b); complete change of surroundings at service and elimination of the cause of pain (c); or by using a mixture of stimulating and inhibiting stimuli (as would occur under natural conditions), leading the bull to the cow, but not allowing him to try to mount and then afterwards allowing him to do so (d). It has been found (Lamoreux, 1941) that hens which lay at a low rate (13 to 22 eggs during a 6 weeks period) produce more infertile eggs than those which lay at a more rapid rate; it has been suggested that this is because low producing hens copulate less frequently or that the life of the sperm is shorter in such hens.

A vague condition known as incompatibility was often cited by the older writers as a cause for sterility. While much of this may have been due to lack of spermatozoa or other conditions given above, it has been suggested by Guyer (1921), McCartney (1923), Walsh (1925) and Pommerenke (1928) that spermatoxins produced in the female are concerned ; but no very convincing experimental work to show that this condition operates in nature to produce sterility is available (but *see* p. 707). While in fowls high titres of spermatozoal antibodies can be produced in the blood stream, infertility is not produced by this (Lamoreux, 1940). Intracervical survival tests of spermatozoa in man made 2 to 15 hours post coitus have failed to permit of an estimation of the degree of fertility (Williams and Simmons, 1942a). These authors (1942b) have outlined clinical methods for the diagnosis of human sterility. It has been stated, in connection with the use of certain methods of contraception, that the absorption of the semen from the vagina exerted a beneficial influence on the female reproductive organs ; no effects of this nature, however, could be found by Bacsich *et al.* (1945). An account of the causes, diagnosis and treatment of impaired fertility in women has been given by Siegler (1945).

Artificial Insemination

The first scientific experiments on artificial insemination were those of Spallanzani (1785) on the dog ; the early literature has been summarised by Iwanow (1907). It is said that long ago artificial insemination was used by Arabs who inseminated mares by semen absorbed on cotton (Gautier, 1905 ; Curot, 1908). Up to about 1920 most of the work done in this field was undertaken to cure certain forms of sterility in man, such as hypospadias (Hunter, described by Home, 1799 ; Sims 1866) and in domestic animals, such as an undilated cervix in mares (Heape, 1897, 1898). With the development of new techniques, however, in recent years, artificial insemination has come to play an important part in normal breeding operations in domestic animals, particularly in dairy cattle. This is because it enables so many more females to become impregnated by a male of outstanding genetic worth, for example by a " proven bull," one which has already bred daughters producing high milk yields. Some 342,000 dairy cows were bred in this way in U.S.A., and some 400,000 in Denmark, in 1945, while in U.S.S.R. (Milovanov, 1934, 1938a ; Neumann, 1935) very large numbers of horses and sheep in addition to cattle have been bred by artificial insemination. In species which normally pair off, such as the fox, the number of males required can be reduced by artificial insemination (Starkov, 1937). Methods of preserving the life and fertilising power of the sperm outside the body have also been developed so that semen can be transported long distances by air and conception obtained from parents separated in some species (but not yet in others) by thousands of miles (*see* p. 698). Artificial insemination has also proved useful in preventing the spread of certain venereal diseases such as dourine in horses and trichomonas infection in cattle (Siebenga, 1938). Another use to which it has been put is for making crosses between animals belonging to varieties in which the disparity in size is so great that coitus between them is difficult or impossible. Plönnis (1876) inseminated a lap-dog with the semen of a setter and obtained a pup which in most points resembled its father. Albrecht (1895) performed a similar experiment and

obtained a similar result. Millais (*see* Heape, 1897) inseminated bloodhounds with spermatozoa obtained from the Basset hound (a much smaller breed) and obtained litters of cross-bred pups. Recently, reciprocal crosses have been made between the large Shire horse and small Shetland pony (Walton and Hammond 1938); in these the size of the cross-bred foal differs according to the size of the dam (*see* Fig. 21. 13).

Crosses can also be made between different species where there is difficulty in getting normal matings to take place. Attempts to cross the hare with the rabbit, however, have been made by this method without success (Yamane and

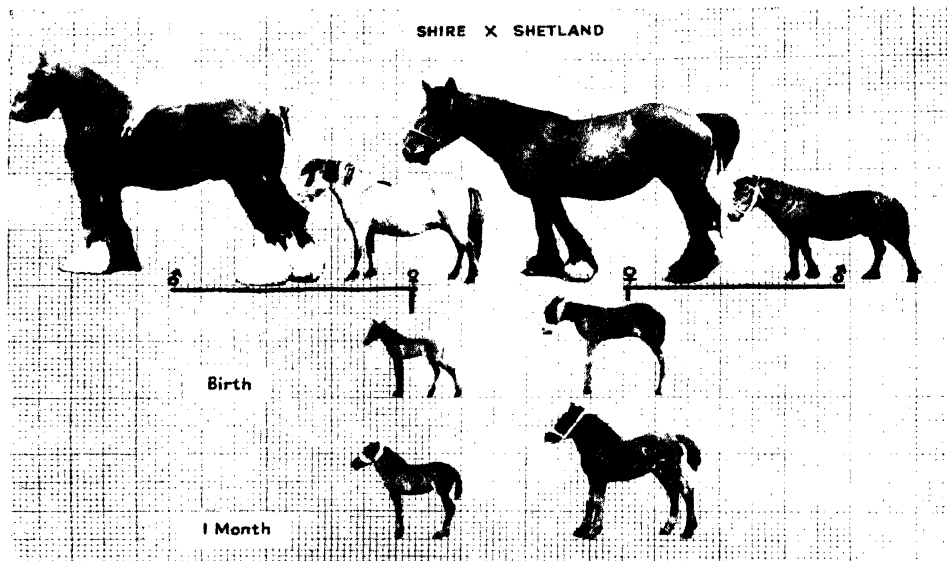


Fig. 21. 13—Reciprocal crosses made between the Shire horse and small Shetland pony by artificial insemination, showing the maternal effect on the size of the foal. *Top line*—Parents: Shire stallion $\times$ Shetland mare, and Shire mare $\times$ Shetland stallion. *Middle line*—Their respective foals at birth. *Bottom line*—The foals at 1 month old. (From Walton and Hammond, 1938.)

Egashira, 1924; Hammond and Walton, 1929). Iwanow (1912) claimed to have inseminated a white mouse with the spermatozoa of a rat, the two offspring produced being intermediate in size; he also crossed horses and zebras. Attempts were made to hybridise many species by means of artificial insemination at Askania-Nova (Serebrovsky, 1935); among the successes already achieved are crosses between the Ural and the domestic sheep (Rumjantsev *et al.*, 1935). Artificial insemination has also been used in unsuccessful attempts to control sex by centrifuging rabbit sperm (Lush, 1925), and in the future it will probably be used in attempts to modify other genetic characters of the sperm by treatment before fertilisation.

The older methods used in the collection of the semen, by sucking it up from the vagina of the female after service and by collection from sponges or gelatine capsules inserted in the vagina are described by Iwanow for domestic

mammals (1912) and birds (1913b). These have now been superseded for the most part by new methods developed in recent years. It is due mainly to the discovery of these new methods that the practical use of artificial insemination has been extended to the field of normal animal breeding. These methods have the advantage that the semen can be collected *in toto* and free from contamination.

In the artificial vagina method (Kusnetzowa *et al.*, 1932 ; Milovanov, 1934 ; 1938a ; Neumann, 1935 ; Sørensen, 1937, 1938 ; Walton, 1938a ; Lambert and McKenzie, 1940 ; Green *et al.*, 1942 ; Anderson, 1945 ; Perry *et al.*, 1945 ; Bonadonna and Bovati, 1946) a cylinder with an inner rubber tube doubled over at the ends is used (Fig. 21. 14— for the bull). The space between the cylinder and tube is filled with water at body temperature and the inside rubber lubricated with pure vaseline. This is held against the flank of the female so that as the male mounts he makes his service into it (Fig. 21. 15). The semen is collected into a glass tube inserted at the end. Various modifications of this apparatus have been devised for different species (*see* references given above). With cows and ewes which have a comparatively short oestrus it is often difficult to find one in oestrus when a collection is required ; in such cases a cow or ewe, not necessarily on heat, may be used, if it is confined in a crate so that it will stand still on the approach of the male (Edwards and Walton, 1938). Many males can be trained to mount a stuffed dummy female, not necessarily of the same species or, indeed, much like an animal at all (Kusnetzowa *et al.*, 1932 ; Walton, 1933 ; Olbrycht, 1935 ; Bonadonna, 1937). Species vary considerably in the extent to which they will use a " dummy " female : while few rams can be used on a dummy almost every boar will respond, bulls and stallions being intermediate in this respect. The natural reflex in a male, as seen in all young animals, is to jump and attempt to serve any animal, but later a conditioned reflex is obtained by resistance

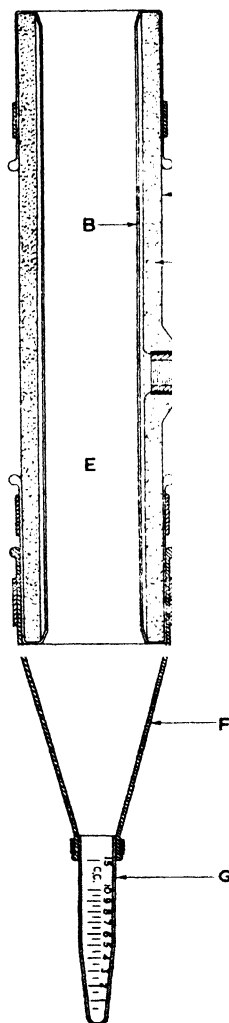


Fig. 21. 14.—Diagram of an artificial vagina for a bull. It consists of a cylinder (A) inside which is fitted a rubber tube (B), the latter being folded over the ends of the cylinder forming a space (C) in which water at body temperature is poured through the screw tap (D). The inner side of the rubber tube (E) is lubricated with vaseline before use. On one end of the cylinder is fitted a soft rubber cone (F) into which is fitted a graduated glass tube (G) to catch the semen when the artificial vagina is held vertically after ejaculation.

on the part of the female not on heat. This resistance varies in different species and so does the ease with which this conditioned reflex is lost when collection is made by an artificial vagina using a " dummy " female. An apparatus consisting of a soft rubber tube has been devised for collecting from the boar by McKenzie (1931) ; in this species a pulsating motion is required to cause ejaculation. An electrical stimulation method used by Moore and Gallagher (1930) on guinea-pigs has been

adapted by Gunn (1936) to rams. Whereas Moore and Gallagher stimulated the medullary region, Gunn stimulates the cord between the 4th lumbar and 1st sacral vertebrae by placing one electrode up the rectum and the other in the back muscle so that the shortest course for the current is through the cord (*see* Fig. 21. 16). After a few, 5 to 10 second, rhythmic stimuli of a 30 volt 50-cycle alternating current, semen is discharged from the penis and collected in a glass tube. Improved stimulators for small mammals have been described by Benham and Enders (1941),



Fig. 21. 15—Method of collecting semen from the bull by means of any early pattern artificial vagina by a left-handed operator. The cow, not necessarily in oestrus, is confined in a crate. As the bull jumps the cow the opening of the artificial vagina is placed over his penis so that he serves into it. (From Hammond, 1940.)

Collery (1944) and others. Another method (*see* Fig. 21. 17) for use with bulls (Miller and Evans, 1934) is to insert the hand in the rectum and gently massage the vasa deferentia; semen is then discharged from the penis without erection. This system has many advantages for if the vesiculae seminales are massaged first their fluids are discharged independently and thereby not only wash out any traces of urine from the urethra, but also ensure that the spermatozoa are obtained in greater concentration (*see* p. 697). Methods for collecting semen from birds have been devised and described by Nikitin (1932), Bernstein (1933), Tinyakov (1933), Warren and Scott (1935), Serebrovsky (1935) and Grecka (1935), while an electrical method has been used by Serebrovsky and Sokolowskaja (1934) and a very effective massage method for cocks has been described by Burrows and Quinn (1935, 1937) and Griffini (1938). After collection of semen it is usually diluted

before being used. There are several reasons for doing this, firstly to enable a larger number of females to be inseminated, secondly to enable the sample to keep better by adding buffer salts to keep the pH constant, and thirdly to reduce temperature shock on cooling for storage. As the semen in artificial insemination is injected directly into the cervix (or in some species—cow—directly into the uterus) many fewer sperms are necessary than in a normal mating where the semen

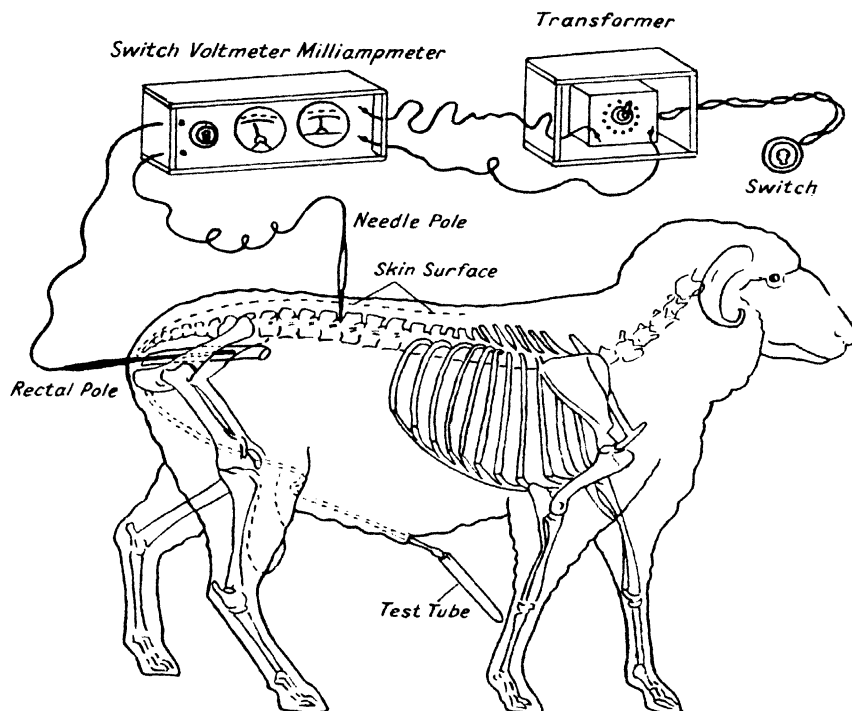


Fig. 21. 16—Diagram illustrating the method of collecting semen from the ram by means of electrical stimulation. (From Gunn, 1936.)

is sprayed over the surface of the vagina and only little of the ejaculate finds its way into the uterus. In early experiments the semen was diluted with isotonic buffered salt solutions only, the composition varying with the species (Milovanov, 1934) and it was found possible to inseminate on the average about 2 sows, 8 mares, 10 cows and 35 ewes from one ejaculation of their respective males (Milovanov, 1934, 1938a; Neumann, 1935; Walton, 1936). Experience indicated, however, that over-dilution was harmful to fertility and storage and so the recent tendency has been to inseminate with very small amounts of undiluted semen in some species, and in other species to add egg yolk to the buffer salt solution. In sheep and goats in which the semen is very concentrated a micro-inseminator (Fig. 21. 18) has been produced for placing very small amounts of semen in the cervix (Milovanov, 1938a). Thus Polovtseva and Fomenko (1936) find that in goats only 0.05 cm.³ of semen or less, if it contains 63 million sperm, gives results

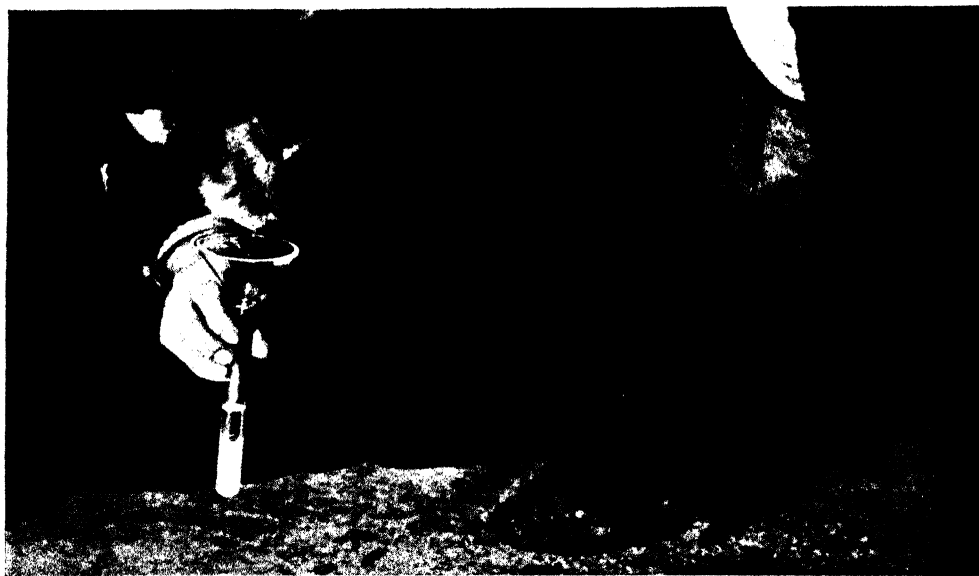


Fig. 21. 17—The massage method of collecting semen from a bull. With the hand in the rectum the seminal vesicles are first massaged and their contents emptied. The ampullae of the vasa differentia are then stroked and the ejaculate, which occurs without erection, is collected in a test tube held under the penis. (From Miller and Evans, 1934.)

equal to natural service : this makes it possible to fecundate a flock of 1,440 she-goats by one male. The number of females that can be inseminated from each ejaculate varies indirectly with the normal dilution of the spermatozoa by accessory fluids—thus the semen of the boar is large in quantity (about 250 c.c.) but the sperm are not dense (about 512 thousand per mm.³), whereas the semen of the ram is small in quantity (about 1 c.c.) but the sperm are very dense (about 4 million per mm.³). Thus while up to 2,600 ewes have been successfully impregnated from one ram in one season (Neumann, 1935), 30 to 40 ewes being



Fig. 21. 18—Micro-inseminator for sheep, consisting of a glass syringe the plunger of which is controlled by a clasp (1) attaching it to a wormed rod (2) held in place by another clasp (3). Movement of the screw (4) thus limits the movement of the plunger so that only a small drop of semen is forced out into the cervix. (From Milovanov, 1938a.)

inseminated from each ejaculate, in the pig only about 2 to 4 sows can be inseminated from each ejaculate. Species differences are illustrated by the following table from Perry *et al.* (1945) :—

Species	Av. vol. of ejaculate (c.c.).	Av. density (millions per c.c.).	Storage survival time (days).	Number of females per ejaculate
Bull	4-5	300-2,000	4-5	40-50
Ram	1	800-4,000	5-6	30-40
Stallion	50-100	50-200	$\frac{1}{2}$	8-12
Boar	200-250	25-1,000	$\frac{1}{4}$	2-4

Experiments by Salisbury (1946) with bull semen indicate that it may be possible considerably to increase the dilution of semen with citrate egg-yolk diluter without lowering its fertility. The figures in the table above relate to a 1 : 10 dilution of semen whereas Salisbury's results are based on dilutions of 1 : 50 and 1 : 100. With collections from bulls occurring once weekly the possible number of females inseminated would amount to 2,300 (at dilution of 1 : 10) per year, but this, in fact, is never realised in practice. On the average at the present time it is possible to impregnate about 1,000 cows a year from one bull. One reason for this is that in natural services with cattle it takes about 1.8 services to obtain a conception, or put in another way, about 60 per cent. of first services result in a conception. Most of those who have used modern methods of artificial insemination in routine breeding practice have found it to be as effective as, or slightly better

than, natural mating (Bartlett, 1937; Sørensen, 1937; Yamane, 1938). In considering conception rates obtained by artificial insemination they should always be compared with the natural rates for the same species, for these are by no means 100 per cent. Even under the best conditions where the bull is run out with the cows it is found that on the average 1·81 heat periods elapse before the cows become pregnant (Rhoad, 1944). In dairy herds in New Zealand only 65 per cent. of cows conceive to the first service (Hale and Courtney, 1940).

Methods which have been developed for the preservation of the life of the sperm outside the body have enabled the semen to be transported long distances (by air and otherwise) and successful impregnation accomplished. Carrier pigeons are, for example, being used in the U.S.S.R. for transport from a breeding centre to distant farms (Milovanov, 1938a). In experiments dealing with the life of the sperm outside the body a distinction must be made between their life as judged by their power of fertilising ova and their power of motility, for just as in other cases mentioned above (p. 678), the latter extends beyond the former. The earlier experiments on the survival of the sperm outside the body for the most part tested the motility only. Iwanow (1926) showed that the spermatozoa of the rabbit may survive for as long as 7 days and those of the guinea-pig 8 days within the removed epididymis if the latter be kept at a temperature a little above freezing-point. Ochi (1916), Sato (1916), and Yamane and Kato (1928a, b) tested artificial media consisting of physiological salt solutions plus glucose for horse and rabbit sperm, while MacLeod (1941a, b) for human sperm found added substrate in the form of utilisable sugar essential for prolonged activity. Wolf (1921) working with rabbits' semen realised the importance of keeping the reaction of the fluid as near that of the blood as possible and used "buffer salts" for this purpose; he was able to maintain the potential motility of the spermatozoa for nine days. Walton (1930a) and Hammond (1930) determined the conditions necessary for the maintenance of the fertilising power of rabbit spermatozoa. Hammond collected the semen from the vagina of the doe after service and tested out its survival at different temperatures, while Walton collected the sperm from the vas deferens of the buck under liquid paraffin so that it was sterile, free from mixture with the secretions of the accessory glands and retained its normal gaseous equilibrium: its survival was then determined at different temperatures. The maximum length of time the fertilising power was maintained under these conditions was as follows:—

SURVIVAL OF FERTILISING POWER OF RABBIT SPERM (HOURS).

Temperature °C	35°	10°	
From vagina—			
exposed to air (Hammond,			
1930)	14	96	16
From epididymis—			
under paraffin (Walton,			
1930a)	14	168	60

The length of time that fertility of the semen is able to be maintained outside the body varies greatly in different species (Anderson, 1945). It varies with the proportion of the sperm to accessory gland fluids in the semen (Hammond, 1936b), so that recent attempts at preservation of the sperm have been directed to the elimination of these secretions. Gunn (1936), for example, found that by collecting the middle fraction of the ejaculate in rams by the electrical method he was able to keep the sperm alive up to 28 days at 4° C., as compared with an average of 12 days for the ejaculate as a whole including the accessory secretions.

Walton (1938b) has been able to double the length of time horse sperm remains fertile by centrifuging the sperm away from the accessory fluids after collection of the semen. In the boar, in which ejaculation occurs in 3 to 5 phases, some phases contain a higher proportion of sperm than others and in these fractions the sperm maintain their motility longest (McKenzie *et al.*, 1938). Whether the accessory secretions shorten the life of the sperm by increasing its activity as such or through their effect on the protoplasmic drop (*see* p. 677) is not known. Nesmeianova (1936) finds in the boar that while the sperm in the epididymis are comparatively low in electrolytes, the content in these is raised, particularly in sodium, during ejaculation by secretions of the accessory glands. Reduction of the amount of accessory gland flow in the semen of the boar by the implantation of stilboestrol tablets failed to increase the life of the sperm in the ejaculate (Wallace, 1949).

The development of the method of storing semen under liquid paraffin at low temperatures led to considerable increase in the length of survival of fertilising power outside the body. By these methods living young have been produced from spermatozoa which had been stored up to about 5 days in the sheep, 3 days in the cow, 12 hours in the horse and 6 hours in the pig (Walton, 1936), while Winters (1937) and Winters *et al.* (1938) were able to obtain lambs from sperm stored for 6 days in the laboratory. By the use of an egg-yolk—glucose—Rochelle salt diluter pregnancies have been obtained with horse and jack semen stored for 24 and 48 hours (Berliner, 1942). The time motility can be maintained in the sperm is, however, much longer; for example, in ejaculates collected from rams by the electrical stimulus method and from animals which had previously been injected with pituitary emulsions, motility of the sperms was maintained up to 64 days outside the body (Gunn, 1936). This time is comparable with the duration of motility in the epididymis (*see* p. 678).

Experiments on the cooling of semen for preservation of the life of the spermatozoa led to the discovery of temperature shock which was liable to destroy the fertility of the sperm. Birillo and Pukhaisky (1936) found that rapid cooling of bull and ram sperm to 0° C. caused temperature shock and advised gradual cooling. This was also confirmed by Gladcinova (1937) and Chang and Walton (1940); Chang (1943) also found that application of ice to the scrotal testes causes disintegration of the epididymal spermatozoa. Phillips (1939) and Phillips and Lardy (1940) showed that egg-yolk, if added to buffered solutions, increased the length of time sperm retained their fertilising power. Chick embryo diluter has but little advantage over egg-yolk diluter (Salisbury *et al.*, 1945); Lasley and Bogart (1944) for boar sperm and Lasley and Mayer (1944) for bull sperm find that temperature shock affects ejaculated sperm more than epididymal sperm. These differences, however, can be eliminated by the use of egg-yolk phosphate

diluter. They (Mayer and Lasley, 1945) have isolated a substance from egg-yolk which gives this resistance to sperm. The optimum rate of cooling varies with the temperature at which the storage is to take place; thus Willett and Salisbury (1942) found for bull sperm that the optimum rate of cooling for storage at 1° C. is 5° C. per 2 hours, but for storage at 5° C. it is 5° C. per 20 minutes. For field use with cattle both yolk-citrate and yolk-phosphate diluents give good results, the citrate being slightly superior to the phosphate when the sperms have been stored 6 days or more (Salisbury, Fuller and Willett, 1941). No observable differences are found in the maintenance of fertility for dilution rates varying from 1 : 2 to 1 : 32 during the first four days of storage (Salisbury *et al.*, 1943). The optimum temperature for storage of the semen would appear to vary slightly in different species and perhaps even more so with the initial treatment of the semen (collection under paraffin, dilution with egg-yolk, etc.). Thus while Komarov and Gladztzinova (1937) find that the survival of bull sperm is longest at 8–12° C., Milovanov *et al.* (1939) find that ram sperm retains its fertilising capacity best at 17–18° C. although Moore and Mayer (1941) arguing from the effect of temperature upon motility and glycolysis rate suggest that 0° C. is the most favourable temperature for the storage of ram semen. With fowl sperm, Grodzinski and Marchlewski (1935) found that motility decreases rapidly at 37° C. In most diluters the optimal conditions for motility were retained at 2° C., but in egg albumen at 16° C. At room temperature, about 23° C., Ishikawa (1930) found that cock's sperm survived longer in egg-white than in physiological salt solution. Reducing the temperature to below 5° C. will destroy the fertilising power of fowl sperm within a few hours even though the motility of the spermatozoa is still maintained (Perry *et al.*, 1945). A satisfactory method for preserving the fertilising power of fowl sperm has not yet been discovered. Fowl spermatozoa have remained viable for 52 days and 30 per cent. resumed motility after having been partially dehydrated by the addition of levulose, quick-frozen to –76° C., and thawed at 42–45° C., but no fertile eggs have been produced after artificial insemination. Chicks have, however, been produced from semen which was quick-frozen at –6° C. and stored for ½ minute, but none after 12 hours' storage (Shaffner, Henderson and Card, 1941). Human spermatozoa regain their motility to the extent of 67 per cent. after plunging them into liquid nitrogen at –195° C. and later rapidly warming them to 35° C., but sperms of rat, mouse, guinea-pig, rabbit and bull do not (Hoagland and Pincus, 1942). Shettles (1940) kept samples of human sperm at –79° C. up to 70 days: no data on fertility of such sperm have, however, yet been obtained.

Several experiments have already been made to test out the practicability of the long distance transit of semen, and with the new methods which have recently been developed many more may be expected in the near future. Young have been obtained from rabbit semen sent by post from Cambridge to Edinburgh (Walton, 1926); from ram semen sent by air mail from England to Poland (Prawochéński and Walton, 1935; Walton and Prawochéński, 1936), from Dubois to Moscow, Idaho, some 700 miles taking 22–50 hours (Terrill and Gildow, 1938) and from Washington, D.C., to Idaho some 2,600 miles taking 31 to 200 hours (Phillips, Schott and Gildow, 1938); from bull semen between Holland and England (Edwards, Walton and Siebenga, 1938; Siebenga, 1938) and, as reported by Bonadonna (1938) from Washington, D.C., to Buenos Aires, Argentina, a distance of some 6,200 miles and taking 7 days. Calves have also been born from

Jersey semen flown across the Pacific from U.S.A. to Victoria, Australia (Elder's Weekly, 1947). Methods of packing semen for long distance transport have been described by Salisbury (1943) and Swanson and Herman (1944).

Various methods have been devised for inseminating the female. In general, inseminating into the vagina gives poorer and more variable results than does insemination into the cervix or uterus. In some species such as the ewe with a long narrow cervix uterine insemination is not possible, but in mares and cows most workers now agree that the uterine method gives the best results with liquid semen owing to the chances of a material portion of the fluid running back into the vagina in the cervical method. It is for this reason that a small undiluted drop of semen is inserted in the cervix of ewes by a microinseminator (Fig. 27. 18). To overcome this difficulty in cervical inseminations in cows, Milovanov (1938b) devised a method of mixing the semen as collected with purified gelatine at body temperature; this is then drawn up and allowed to solidify in small tubes. These can then be inserted into the cervix and the sperm are liberated as the gelatine melts. This method has been widely used in the U.S.S.R. during recent years. Sørensen (1946) has adopted a modification of this method in which the tube of gelatinised semen is placed in a holder and forced into the cervix by the release of a spring: this method is widely used throughout Denmark. In U.S.A. and U.K. where dilution with egg-yolk phosphate or citrate is in general use, uterine insemination is generally practised. The gelatine method has the disadvantage that the effective life of the sperm is limited to about 2 days, whereas with the fluid yolk-phosphate method the effective length of life is about 4 days.

As previously pointed out (p. 680) the time at which insemination is made in relation to the time of ovulation is important. In species such as the cow which ovulate after the end of oestrus, insemination after the end of oestrus enables conception to occur when it would otherwise not be possible, while if insemination is made too early in oestrus (and so too long before ovulation) the sperm are liable to die before the ovum is shed. Thus, for cows, Trimberger and Davis (1943) found that the percentage conception rate from a single insemination was as follows: beginning of oestrus 44, middle of oestrus 82, end of oestrus 75, six hours after end of oestrus 62, twelve hours after 12, thirty-six hours after 8, and forty-eight hours after 0, ovulation occurring on the average about 14 hours after the end of oestrus (Nalbandov and Casida, 1942) and varies from about 10 to 15½ hours post-oestrus (Hamilton and Laing, 1946). Laing's (1945b) results confirm these findings, inseminations made more than 16 hours before the end of oestrus not being fertile. Vighenzi (1943) (*see also* Bonadonna and Bovati, 1946), however, states that no noticeable reduction in the percentage of pregnancies was observed up to the third day from the onset of heat, oestrus length in the cow being about 20 hours. In mares, Laing (1943) and Davison (1947) concluded that the survival time of stored sperm in the female tract was somewhat less than 24 hours and suggested that inseminations should be made 24 hours after injections of P.U. to cause ovulation. By using a urine test on mice to indicate the time of ovulation in women, Farris (1946) finds that the time when insemination is most likely to produce pregnancy is the 3rd or 4th day of reaction to this test. Vimeux (1946) stresses the importance that the day of insemination should coincide with the period of ovulation in woman, and gives various tests for this, including the period of glairy cervical fluid occurring between the 11th and 15th day of the cycle.

IV. THE NUMBER OF EMBRYOS DEVELOPING TO BIRTH

During the course of pregnancy in mammals and incubation in birds, many factors come into play to prevent the development of the embryo. In the mammal, defects in the mechanism of attachment, and later in the supply of those hormones necessary for the maintenance of pregnancy, may lead to absorption or abortion of the young, while in both mammals and birds lethal genetic factors may cause death of the embryo before birth or hatching. In certain special cases the number of young produced at birth is greater than the number of eggs fertilised. In the armadillo (Stockard, 1921; Hamlett, 1933), divisions in the egg after fertilisation lead to the production of four embryos. In other species this phenomenon is the exception rather than the rule, although cases of identical twins, that is, twins derived from a single fertilised ovum, are by no means uncommon in certain species of mammals. The evidence for this is based for the most part on the mirror images of physical character in the twins. Such cases have been described in man by Newman (1917, 1923), and Newman *et al.* (1937), and in cattle by Gowen (1922), Lush (1929), Hutt (1930), Kronacher (1936) and Bonnier and Hansson (1946). In the marmoset monkey, of whose reproduction twinning is a special feature, Wislocki (1932) inclines to the view that the twins are as a rule derived from a single ovulation. In this connection it is perhaps significant to note that those who have investigated the genetic history of multiple births in man (Davenport, 1920; Greulich, 1934, 1948) find that the records of twinning are quite as marked, or even more so, on the father's side of the pedigree as on the mother's side.

Failure of Attachment

In most species the fertilised eggs take 3-4 days to travel down the tubes and pass into the uterus. If oestrogen is injected into mice and rabbits during this period, however, the blastocysts become "tube-locked," and perish (Burdick and Pincus, 1935; Pincus and Kirsch, 1936; Whitney and Burdick, 1936; Burdick, Whitney and Pincus, 1937). The tubal journey of ova is shortened on the other hand by about 12 hours in pseudo-pregnant rabbits in which a second ovulation has been induced by gonadotrophic hormone (Wislocki and Snyder, 1933).

In order that implantation of the blastocyst shall occur the uterine mucosa has to be in a receptive state: Allen and Corner (1929) among others have shown that this receptive state is brought about by progesterin secreted by the corpus luteum (*see* Chapter 11). Under certain conditions this secretion is absent or deficient in amount and so leads to failure of the fertilised ova to become implanted. The condition may be temporary only, as occurs in the rat and mouse during a certain period of lactation (King, 1913; Kirkham, 1916), and merely causes a prolonged pregnancy or may be more lasting, as in the rabbit (Hammond and Marshall, 1925), and cause lack of implantation or atrophy and absorption of the developing embryos. Brambell (1937) is of the opinion that failure of the blastocysts to develop may be due to lack of a growth-stimulating substance in the uterine lumen. In the cow, coincident lactation does not interfere with normal development of the young. In sheep, Litovchenko (1936) finds that suckling during the first part of the pregnancy period has no ill effects on the embryo:

his sheep were brought into heat in June by injections of pregnant mare serum while still suckling. The failure to obtain pregnancy after injections of gonadotrophic hormones during the cycle in cows and in sheep (Zawadowsky *et al.*, 1934a, b) may be due to the fact that the corpus luteum already in existence and not the newly formed corpus luteum regulates the cycle (Zawadowsky and Eskin, 1937). Failure of implantation would therefore occur because the uterine mucosa was not in the receptive stage. The exact duration of the time over which implantation is possible in different species needs working out (*see* Chapter 16). After squeezing out the corpus luteum in the cow at any stage of the cycle ovulation occurs in the course of a few days and pregnancy ensues from matings ; but when ovulation is caused by the injection of gonadotrophic hormones without the removal of the corpus luteum the cows fail to conceive ; this also occurs in sheep (Zawadowsky, *et al.*, 1937). It is probable that the uterine changes concerned with implantation are rightly timed when the corpus luteum is removed, but are not when a cyclic corpus luteum is already in existence. In rabbits injected with prolan on the 7th day of pseudo-pregnancy and inseminated, Wunder and Padootcheva (1936) failed to obtain pregnancy and attributed the failure to prevention of the sperm-passage up the tract ; they failed, however, to consider the possibility of lack of implantation as the cause of the sterility. By injecting folliculine-benzoate along with the pregnant mare serum into cows, Zawadowsky and Eskin (1937) found that the uterine mucosa was prepared and the obstacle for conception was removed. For attachment a proper relation between progesterone and oestrogen, balanced on the progesterone side, is required, for when additional oestrogens such as ethinyl oestradiol and diethyl stilboestrol are given by mouth they prevent implantation of the blastocysts in rabbits and rats (Parkes *et al.*, 1938).

Absorption or Abortion

Injection of oestrogen given during early pregnancy will over-ride the action of progesterin from the corpus luteum and so lead to a failure of the ovum to form an attachment to the uterus ; if given later in pregnancy oestrogen will cause absorption or abortion in rats, mice, rabbits and cats (Smith, 1926 ; Parkes and Bellerby, 1926 ; Courrier and Kehl, 1933 ; Courrier and Raynaud, 1934 ; Courrier and Gros, 1935b ; Robson, 1935a, b). The amount of oestrogen required to produce abortion in rabbits decreases as the period of pregnancy advances (Raynaud, 1934). Abortion or absorption of the young in the rabbit is also caused by injections of anterior pituitary extract during pregnancy (Bellerby, 1935) ; but this not invariably occurs with prolan injections (Hill and Parkes, 1932a). Reynolds (1937) finds that the disproportions between the growth increments of the foetus and uterus during the last part of pregnancy are due largely to the increase in the influence of oestrogen with the advance of pregnancy ; as these disproportions are concerned with the onset of labour, abnormal rises in the level of oestrogen in the blood may account for cases of spontaneous abortion. Possibly related to this is the fact that in the horse, a species in which only about fifteen twin births per thousand occur (Lauprecht, 1932), more than one-half of the twin pregnancies end in an abortion (Wriedt, 1928). Removal of the ovaries or destruction of the corpora lutea during the early stages of pregnancy also leads to atrophy and absorption of all the embryos (Fraenkel, 1903 ; Weymeersch, 1911 ; Hammond and Marshall, 1925 ; Courrier

and Gros, 1935a). The effect of removal of the ovaries during the latter stages of pregnancy after the placenta has been fully formed varies, however, in different species. In those in which the corpus luteum persists throughout the whole of pregnancy, for example in the goat (Drummond-Robinson and Asdell, 1926), cow (Hess, 1921; Schmaltz, 1921), rabbit (Kleinhaus and Schenk, 1908; Hammond and Marshall, 1925) and mouse (Parkes, 1928b; Robson, 1935a), abortion invariably results. In woman, however, in which the corpus luteum disappears during the middle of pregnancy, removal of the ovaries during the latter part of pregnancy does not necessarily cause abortion (Bell, 1920; Essen-Möller, 1904; Ask-Upmark, 1926). It seems possible also that the mare would behave similarly, for no corpora lutea exist in the ovary during the latter part of pregnancy, but experiments have not yet been made on this species. The guinea-pig (Daels, 1908; Herrick, 1928) is also known to behave in this way; in the cat, too, removal of the corpora lutea or ovaries after the 49th day does not interrupt pregnancy (Courrier and Gros, 1935a). In the sheep, Casida and Warwick (1945) have established the fact that in at least some cases the ewe can maintain pregnancy without the presence of the corpus luteum after the 55th day of gestation. It is believed (Selye, Collip and Thomson, 1935; Haterius, 1936) that progesterin is also secreted from the placenta as well as from the corpus luteum, for in animals such as the rat pregnancy may be maintained after removal of the ovaries shortly after mid-pregnancy if all but one of the foetuses are removed, provided the additional placentae (of the removed foetuses) are allowed to remain *in situ* (see Chapter 16). During the second half of gestation the anterior pituitary appears to have no action in maintaining pregnancy. Walton and Hammond (1934) found that in ferrets pregnancy could be maintained in the non-breeding season at a time when the gonadotrophic activity of the anterior pituitary was absent or reduced. Moreover, hypophysectomy made during the second half of pregnancy in the rat (Pencharz and Long, 1933), mouse (Selye *et al.*, 1933), cat (Allen and Wiles, 1932), guinea-pig (Pencharz and Lyons, 1934) and ferret (McPhail, 1935) has shown that pregnancy can continue uninterrupted for its normal duration in the absence of the anterior pituitary. Hypophysectomy, however, in the early stages of gestation quickly leads to termination of the pregnancy, probably through its action on the activity of the corpus luteum (see Chapter 12). Although in many species the corpus luteum persists throughout the whole of pregnancy it is only in the early stages that the uterus displays those changes which make it receptive to the attachment of the blastocyst. Heape (1890) was the first to show that the fertilised egg of one rabbit could be transplanted to another at this early stage of pregnancy, while Pincus (1930) found that transplantation could also be made into the pseudo-pregnant doe if it was done at the appropriate stage of the cycle. Fertilised ova have also been successfully transplanted in both the sheep and the goat (Warwick, Berry and Horlacher, 1934). Chang (1947, 1948) has shown that rabbit ova in the two cell stage can be stored at 10° C. for four days outside the body and produce normal young after transplantation into the Fallopian tubes of does fourteen hours after ovulation. The conditions necessary for the proper development of the blastocyst are now being worked out by growing the fertilised ovum by tissue culture methods (Lewis and Gregory, 1929; Pincus, 1936), and these, when known, should throw some light on the possible causes of the failure of the ovum to develop. It is not yet known, for example, whether

the "cervical stem or rings" method of contraception (Dickinson and Bryant, 1932), reduces the carbon-dioxide, or raises the oxygen, tension in the uterus above the levels required by the ovum and so causes its death, or whether the results are achieved in other ways. Mares in which part of the reproductive tract has been injured at foaling and which suck air into the reproductive passage are sterile, and these can be cured by suturing the labia over the dorsal commissure of the vulva so that the normal semi-anaerobic state of the reproductive tract is regained; as a result of this, inflammatory conditions of the tract clear up (Caslick, 1937; Day, 1939b). There has been, as yet, little investigation as to how far various pathological conditions of the uterus prevent implantation of the blastocyst.

Abortion results in much loss in fertility among many species of animals. In women, Kelly (1908) estimates that from one in five to one in ten pregnancies end in this way, while Franz (1898) from records of a maternity hospital in Halle finds that it accounts for 15.4 per cent.; furthermore, Williams (1904) expresses the opinion that in private practice every 5th or 6th pregnancy ends in abortion, and that if early cases, in which there is a profuse loss of blood following retardation of the menstrual period, are taken into account it would be much higher. In sheep, Heape (1899b) found that it varied from zero to 24 per cent. in different flocks and averaged 2.4 per cent. Breed differences were found, although as was suggested by Marshall (1908) for Scottish Blackfaced sheep, this is probably due to adverse conditions under which certain breeds are kept. Kelley (1937) suggests that early abortion or resorption of the foetus is one of the factors causing infertility among Australian Merino sheep. Among domestic animals it is with cattle, and particularly dairy cattle, that the greatest losses occur. Heape (1906) estimated this to be not less than 10 per cent. for all breeding cows and is often considerably higher in districts where contagious abortion occurs. Since his time it has become widespread throughout the country and there is hardly a district without it; this spread occurred at a period shortly after the 1914-18 war when the dairy herds in the country were rapidly increasing and milch cows were being transferred from one district to another.

In man deliberate abortion among civilised nations is a criminal offence punishable by law, but nevertheless is not infrequently carried out. In oriental countries and among savages abortion is practised more openly. The more usual means are drugs (ergot, ethereal oil of juniper, yew, turpentine, camphor, cantharides, aloes, etc.) but none is infallible and, owing to their toxic properties their use is often accompanied by danger (Bloch, 1908). Haddon (1908) states that among the Eastern Islanders of the Torres Straits abortion is procured by the leaves of the shore convolvulus and certain other plants; also that when the leaves of argerarger (*Callicarpa* sp.) and bok are eaten and the woman's body is saturated with the juice from the leaves she is believed to be proof against fecundity. Early elective abortions can be caused by curetting the uterine mucosa, by injecting tincture of iodine into the uterine cavity and by X-rays (Dickinson and Bryant, 1932). Abortion is sometimes procured by purely mechanical means, e.g. blows, massage, hot injections, carrying heavy loads, etc. But although mechanical and psychological influences, both voluntary and involuntary, play a part in bringing about abortion, they are probably less frequently concerned in the process than pathological conditions existing either in the embryo or in the maternal organism.

Among the causes of abortion in women may be mentioned (*see* Kelly, 1908) various infections of the mother (notably syphilis), psychic disturbances and excessive cohabitation, and acute poisoning (by alcohol, phosphorus, lead, etc.). It is believed by some (Galabin, 1904) that the incidence of abortions is greatest at the epochs which would have been menstrual periods if pregnancy had not occurred.

Abortion exists also among horses, but there are no figures to show the frequency of its occurrence; it is probably most frequent during the period from the 6th to 9th week of pregnancy, and Ewart (1897) explains this as being due to the fact that at this time the yolk-sac placenta is being replaced by allantoic villi. Thus it may be said that at this time there is a tendency on the part of the embryo foal to revert to the condition of its marsupial ancestor.

Abortion in cows is, according to Wallace (1907), liable to be due to the following:—(1) eating ergotised grass, (2) injury due to horning by other cattle, hunting by dogs, or shaking and bruising in travelling, (3) physical strain resulting from walking over too soft land, etc., (4) very cold or foul water, or frozen turnips, etc., (5) superpurgation, whether occurring naturally or as a consequence of dosing by physic, (6) contagion from other cows infected with contagious abortion.

While in many cases the cause of the abortion may be well known, the mechanism by which the result is achieved is frequently but little understood. For example, while it is known that contagious abortion is due to the infection of the placenta with the bacillus of Bang (1906), and to a minor extent with other organisms such as *Streptococcus* (Wall, 1914; Nielsen, 1926) and *Trichomonas* (Riedmüller, 1933), the mechanism by which this is brought about is but imperfectly known. Cases occur in which, although the infection is present, the embryo is carried to full time while abortions may occur either early or late in the pregnancy period. It is possible that the result depends on the destruction of the placenta affecting the production of the necessary hormones for the continuation of pregnancy, but this is still an unexplored field (Vogt-Möller, 1939). For collecting early blastodermic and embryonic stages for study, the fertilised egg can be washed out of the uterus in monkeys (Lewis and Hartman, 1933), mares (Hammond, 1938b; Day, 1939c) and cows (Petersen and Dracy, 1947). The effects of ergot and related drugs which are considered to cause uterine contractions, and so abortion, have been investigated by Knaus (1927), who found in the rabbit that while adrenalin increased muscular tone at all stages of pregnancy, ergot was, like pituitrin, only effective after the 18th day of pregnancy. Knaus (1926, 1934) found that in animals such as the rabbit where the corpus luteum persists during pregnancy, the presence of an active corpus luteum (up to the 17th day of pregnancy) made the uterine muscle insensitive to injections of posterior pituitary extract, but from about the 18th day when the activity of the corpus luteum declined injections caused contraction and death of the embryos which were aborted 3 or 4 days later; at a later stage still (after about the 28th day) immediate birth of the young was caused. It is possible that many of the naturally occurring causes of abortion (*see* above) act on such mechanisms as these in bringing about their effects, but little work has yet been done in this field. Absorption of the embryos is also caused by lack of vitamin E in the diet (Evans and Bishop, 1923; Mason, 1939). Absence of vitamin G in the diet reduces the hatchability of hens' eggs, mortality occurring mainly between the 7th and 10th days (Davies, Norris and Heuser, 1938).

Foetal Atrophy

Lethal genetic factors in many species may cause death of the embryos at or before birth or hatching ; in such cases some only of the embryos perish, but not all as they do under the conditions quoted above. The finding of unexpected Mendelian ratios when yellow mice were bred together led Kirkham (1919) and others to the belief that the homozygous condition for yellow was lethal. Such lethal Mendelian characters which cause uterine death of the embryo have been described in many species (mouse, Little, 1920 ; Dunn, 1937 ; cat, Jones, 1922 ; horses, dogs, sheep and cattle, Mohr, 1929 ; Wriedt, 1930) ; they have also been found in fowls (Dunn, 1923 ; Hutt and Greenwood, 1929). They are almost all " recessive " in inheritance and it is for this reason that fertility is frequently reduced by inbreeding. For example, on inbreeding guinea-pigs for 25 years Eaton (1932) found a decrease of fertility which was associated with an increase of prenatal mortality. It seems probable that it was on account of a recessive lethal character that the famous Duchess family of Shorthorn cattle bred by Bates eventually became extinct. The " bulldog " calf found in the Dexter breed of cattle is an example of such a lethal character ; this gene in the heterozygous condition gives rise to the short-legged type, while in the homozygous produces the " bulldog "—an abnormal embryo in which length growth of the bones fails to take place and which is usually aborted at the 5th-7th month (Crew, 1923). A somewhat similar abnormality occurs in the Norwegian Telemark breed

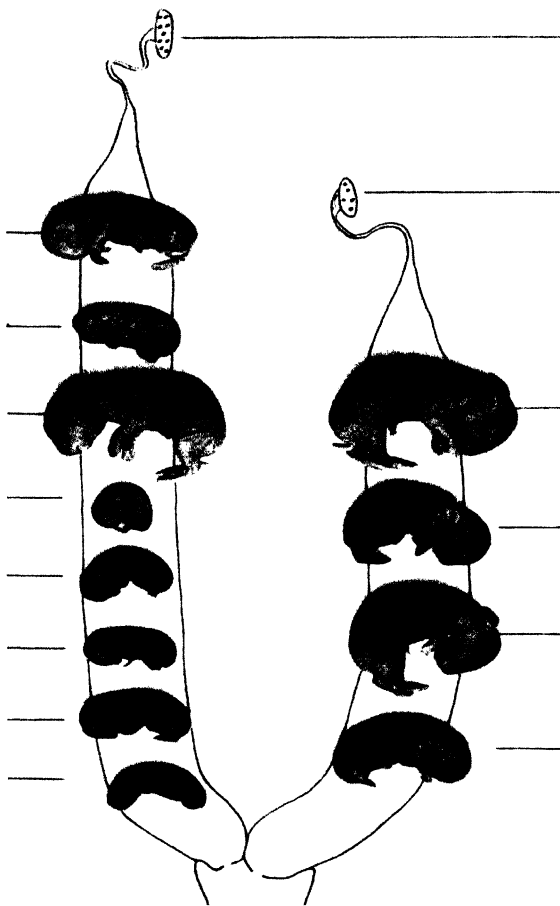


Fig. 21. 19.—Foetal degeneration in a 29-day pregnant rabbit of a strain which is genetically pure for this character. Although 12 ova were fertilised only 3 embryos remain alive, the rest having perished at different stages of development. (From Hammond, 1928.)

of cattle (Wriedt, 1930). In those species which produce only one young at birth, abortion or absorption of the embryo results from the death of the embryo, but in those species in which many young are produced the dead and mummified embryos are carried in the uterus to full term and may be found mixed with the cleansings after birth (Hammond, 1914, 1921). Foetal atrophy is common among pigs, rabbits, rats, guinea-pigs, mice, dogs, ferrets, and cats (Robinson, 1918, 1921; Hammond, 1921; Corner, 1923; Nicol, 1933), and also occurs in cows (Turner, 1924) and man (Streeter, 1933) (*see* Fig. 21. 19). In the wild rat over half the litters conceived suffer some depletion before birth; on the average 10 eggs are shed and $7\frac{1}{2}$ young are born (Perry, 1945). In the rabbit foetal atrophy is inherited as a maternal character as distinct from the homozygous embryonic condition mentioned above. This is shown by the fact that does carrying this character produce approximately the same number of atrophic young no matter whether they are mated with bucks of their own or unrelated strains (Hammond, 1928). The embryos die off at different stages during the course of development and there is some reason to suppose that their death may be caused by the deficiency of some substance in the mother's blood; for if one tube is cut, so as to reduce the number of ova fertilised a much smaller percentage of foetal degeneration occurs than in normal animals where a larger number of ova are fertilised. Some support for this suggestion is also obtained from experiments in superovulation in rats, in which large numbers of ova are fertilised and become implanted, but a large number perish and undergo absorption, the number of young born being almost never in excess of normal (Evans and Simpson, 1940). In these cases foetal atrophy at various stages of development occurs. Such results seem to point to the conclusion that the number of young that develop normally to birth depends on the level of some substance in the blood of the mother which acts as a limiting factor, but what this factor is is not yet known. In old age there is some indication that the proportion of these atrophic foetuses is increased within any one strain, accounting for some of the decrease in fertility which occurs after the prime of life (*see* p. 654). In wild rabbits Brambell (1942, 1944) found that 60 per cent. of all litters are lost before the 20th day of gestation. This enormous mortality occurs chiefly on the 11th and 12th days of gestation after implantation and when the placenta is already well established. Unlike the foetal atrophy described above, however, most of this atrophy is accounted for by the total loss of whole litters. Among women it is well known that some individuals show a tendency to repeated abortions (Vignes, 1923) and it may be that some deficiency of the same nature is involved. Stephenson (1885) some time ago suggested that abortion might be due to lack of vigour in the spermatozoa of certain bulls resulting in reduced vitality in the eggs fertilised by them, and others since have taken this view. Hammond and Asdell (1926), however, could find no evidence for this in rabbits which were inseminated with sperm which had almost reached the limit of their fertilising power, when kept either in the female tract or outside the body. Riddle and Behre (1921), too, state that in ring-doves no evidence was obtained indicating weakness or modified viability in the embryos obtained from stale sperm fertilisations. Dunn (1927), also, in fowls found no evidence of any effect of the age of the sperm on the viability of the embryos. Young and Blandau (1936), however, find in guinea-pigs that the age of the ovum at fertilisation affects the viability of the embryo: this is first expressed when the

ovum is 8 hours old and although the ovum may be fertilised up to 26 hours normal young are not produced from ova more than 20 hours old. As the ova became older so the pregnancies tended to be terminated at an earlier stage. Blandau and Young (1939) suggest that this may be one of the causes of abortion in women. Warwick, Berry and Horlacher (1934) found that when Angora goats were served by rams 21 out of 38 conceived and implantation took place, but the hybrid embryos perished before the end of the gestation period. The cause for this, however, has not yet been determined.

Embryonic mortality in fowls' eggs is not distributed at random throughout the entire incubation period but normally has two peaks centred about the 3rd and 19th days (*see* Fig. 21. 20), and at corresponding periods in pigeons and doves (Riddle, 1930). According to Romanoff (1931) the external factors such as incubation environment have greater effect during the second critical period. Many different types of abnormalities may be found in the developing egg (Hutt and Greenwood, 1929) and several lethal mutations are known which cause death of the embryo. An account of these and the other factors which affect the hatchability of fowls' eggs together with references to the literature has been given by Landauer (1941). The mortality from eggs with defective firm albumen is higher for the entire incubation period, but markedly so during the 2nd to 7th days (Hall and van Wagenen, 1936). Malposition is to a large extent responsible for the fact that embryonic mortality rates are higher in large eggs than in those of medium size (Hutt, 1938). The amount of embryonic mortality (dead in shell) shows consistent differences in individual hens, especially among those of three years old and over (Blyth, 1945).

Insko *et al.* (1947) also found that embryonic mortality increased with the progressive ageing of hens. Recently it has been found that in man death or disablement of the young may be caused by antibodies set up in the mother's blood as a result of incompatibility in certain blood groups (Rh factors) between mother and foetus. These antibodies destroy the red blood corpuscles of a susceptible foetus so that successive offspring from the same parents are progressively affected more severely. There is some evidence also that such factors may be responsible for early and late foetus death (Levine, 1943). The viability of the fertilised eggs of the rabbit is not affected in the early stages of development by their being stored and incubated in heterologous as compared with homologous serum (Chang, 1948).

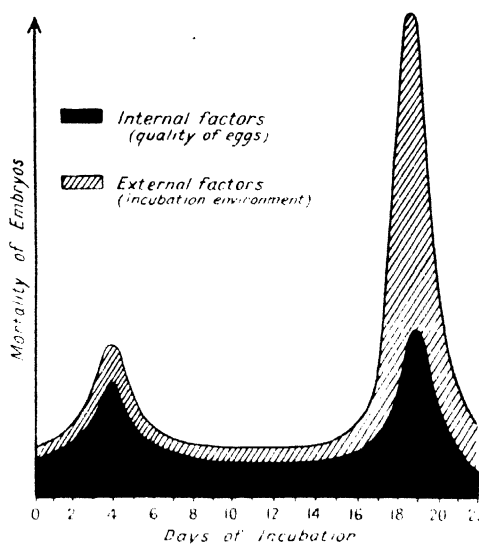


Fig. 21. 20—Frequency distribution of embryonic mortality in incubated eggs. (From Romanoff, 1931.)

V. THE INHERITANCE OF FERTILITY

That fertility is a racial characteristic and consequently is capable of hereditary transmission is a fact that is generally accepted. Among sheep, for example, some breeds like the Dorset Horns, Suffolks and Half-Breds (Border Leicester $\times$ Cheviot) are notoriously prolific while other varieties like the Blackfaced and Southdown are relatively infertile. Moreover, there is a considerable amount of evidence that in each breed there are particular strains of related individuals which have a higher degree of fertility than the average ; this is the case where it has been the practice to breed from twin ewes and rams (Marshall, 1908). In pigs, Stewart (1945b) estimates that heritability accounts for about 17 per cent. of the variability in litter size. In cattle, Hewitt (1934) has given examples of twinning that runs in strains. Much statistical work has been done to obtain scientific proof of the inheritability of fertility ; for example, Pearson *et al.* (1899) investigated it by means of correlation coefficients in Thoroughbred horses, and Rommel and Phillips (1907) in Poland-China sows, while Nichols (1924) found marked differences between various breeds of sheep. On the other hand Pearson (1907) in mice, and Pearl and Surface (1909) in fowls, failed to find a sensible parental correlation in fertility. It has been shown that crosses between a fertile breed of sheep like the Border Leicester and a breed of lower fertility like the Merino give ewes with an intermediate grade of fertility. Thus Colebatch and Scott (1931) in sheep kept under the same conditions found that the Merino gave a lambing percentage of 93 whereas the Border Leicester-Merino crosses produced 111 per cent. of lambs. Evidence for the fact that twinning is inherited in sheep has also been presented by Langlet (1935) and Patow (1933). Pearl (1912) was probably one of the first to apply the method of Mendelian analysis to the problem by postulating various factors of a physiological nature concerned in the number of eggs laid by the fowl, such as for the different levels of winter egg production. He arrived at the theory that high egg production was a sex-linked factor and is transmitted by the cock whose female offspring manifest it in the next generation. While further work in this field (Punnett, 1930) has failed to substantiate the fact of sex-linkage of high egg production there can be no doubt that Pearl did substantially increase the productivity of the Barred Plymouth Rock fowls by the methods which he adopted. His experiments laid the foundation for the "progeny test" system of selection which is used to-day for all commercial quantitative characters and especially for those such as fecundity and milk secretion which cannot express themselves in males. His method of postulating factors for characters of a physiological nature which are concerned in high egg production in fowls has been followed, too, by recent workers. Goodale and Sanborn (1922), for example, divide the characters concerned in high egg production in fowls into those affecting early sexual maturity ; high intensity of production ; no winter pause in egg production ; non-broodiness ; and high persistency of production (*see* Greenwood, 1935). The eggs of some breeds (White Wyandottes) are less fertile (by 10 per cent.) than others such as the White Leghorn or Rhode Island Red, but whether this is due to lack of sperm production or inherited lethal genes is not known (Hutt, 1940).

In mammals the problem is still further complicated by the number of stages in the process of reproduction at which control of fertility may be exerted (*see*

above). Some analytical work, however, has been attempted although at present the "progeny test" still forms the basis of improvement in practice. In rabbits, for example, high fertility is dependent on: (1) the number of ova shed at each heat period; this behaves as a multiple factor character and is intermediate in the first cross from high and low ova-producing strains; and (2) the number of embryos which undergo atrophy before birth: this is a maternal recessive character and segregates out in Mendelian fashion (Hammond, 1934a). A third factor which influences fertility is the number of spermatozoa produced by the male and a complication arises that unless this is optimum the second factor referred to above cannot be measured with accuracy. The fact, frequently noticed, that a female will produce more young when mated to one male than what she will have when mated with another has been explained by the greater viability of the young in utero, especially if they happen to be cross-bred, but recent work (*see* p. 671) has shown that sperm production by the male is equally, if not more, important. This is certainly so in the sheep in which species there is little foetal atrophy. Heape (1899b), for example, who made the observation that Dorset ewes when mated to a Hampshire ram produced more lambs than when mated to a ram of their own breed, attributed this to the greater viability of the cross-bred embryo, but recent work on sperm production makes it necessary that further consideration should be given to the sperm production of the males concerned. In man the literature contains numerous reports of individuals who have had a large number of multiple births (*see* Greulich, 1938); many of the publications point to the existence of a hereditary tendency to the production of fraternal twins. A surprising result, in view of the accepted theory of their genesis from double ovulations, is that the history of twin births seemed to be quite as marked on the father's side as on the mother's (Davenport, 1920; Greulich, 1934). (*See also* p. 700.)

VI. THE BIRTH RATE IN MAN

The world position in population has been summarised by Pearl and Gould (1936) who concluded that the population of the world has been growing in the recent past at the rate of about 1 per cent. per annum. They find that since the middle of the 17th century world population has exhibited an extraordinary spurt of growth, due probably to the expansion of man's effective universe, as a result of scientific discoveries and their application. There is an extreme unevenness of the distribution of the population densities over the earth's surface (*see* Fawcett, 1947): 57 per cent. of the total land area of the earth is occupied by only $4\frac{1}{2}$ per cent. of the total population of the world. Only a little over 5 per cent. of the land area of the earth contains 52 per cent. of its population.

The birth rate figures for man have for years afforded food for thought for the statesman and political economist. It is surprising to find, for example, that, in the United States, of the potential mothers in a biological sense (*i.e.* all women of breeding age), none in excess of 10 per cent. actually became a mother (Pearl, 1936b). Pearl (1937a), from an analysis of parity order by age of mother, concludes that the effect of contraception as actually practised in the population is not having nearly as great an effect in lowering the reproductivity of those women who from year to year are doing the national breeding as many would have us believe. Other factors, notably deferred marriage, play extremely important

rôles. Birth control as actually practised acts in keeping women out of the category of breeders or admitting them into that category only once in a lifetime. Birth rate figures are intimately bound up with the development and social problems of nations and a clear conception of the biological principles underlying them is required for their proper interpretation. Too often in the past legislation has sought a remedy at variance with biological principles and so has proved futile to stem the tide of a falling birth rate.

In this connection Herbert Spencer's (1899) generalisation concerning fertility stands out as the main axis around which minor causes revolve. He suggested (*see* p. 648) that the power to sustain life and the power to produce new individuals are inversely proportional, that is, that Individuation and Genesis vary inversely. This generalisation will equally well explain the variations that exist in the birth rate of man—variations which occur both in time and in space. The East, with the low standard of Individuation generally, of nutrition, education and living, has a higher birth rate than the West, where the great expenditure of time and material on the individual of necessity lowers the rate of Genesis if this standard is not only to be maintained but increased. For example, from low to high standard of living the birth rate decreases in space (1931) from 32.2 in Japan to 24.9 in Italy, 17.4 in France and 14.8 in Sweden (Charles, 1934). Similarly with time, as Individuation progresses with the advance of civilisation, so Genesis and the birth rate fall in order to allow the succeeding generation better facilities for obtaining fuller Individuation. Thus, no matter how the fall in birth rate has been discouraged by bounties on large families and by the prohibition of contraceptives, etc., it has continued to fall in countries in which progress in Individuation is being made; for example, the birth rate in Italy fell from 38.7 in 1860 to 31.1 in 1920, and 24.9 in 1931 (Charles, 1934).

It is now almost a century ago since Malthus (1872) advanced his famous proposition that whereas population tends to increase in geometrical ratio the means of subsistence increase only in arithmetical proportion. As a consequence of the acceptance of this doctrine many political economists tended to see in over-population the most fruitful source of pauperism, disease and crime, and the cause of increasing congestion in the future. That Malthus' predictions have not been verified is a matter of common knowledge and the problem as the modern economist sees it is not so much how to place a check on population generally, but rather how to secure that future generations shall be sufficiently recruited from that section of the population which is industrially capable and at the same time preventing indiscriminate propagation. Here one comes in conflict with Spencer's generalisation, for in the upper classes of society where Individuation is most marked Genesis often tends to be sacrificed in order that Individuation may be fulfilled. Rather the solution of the problem lies in increasing the Individuation in the lower classes of society, or in allowing the community instead of the parents to take over the costs of Individuation.

As has been indicated above there is abundant evidence that in most civilised countries at the present time the birth rate (that is, the proportion of the children born to the population) is tending to decrease, while in some countries (such as in France) the actual population is diminishing. There has, however, been a slight rise in the birth rate in Great Britain in the last three years and, owing to special measures taken just before the 1939 war, a greater rise in Germany and Italy.

Statistical analysis by Newsholme and Stevenson (1906) and Yule (1906) has shown that the observed fall is not simply a consequence of changes in the ages of the people, or in the proportion of married to single women, for allowing for such alterations as have occurred, the number of births per 100,000 of the population in England and Wales dropped from 3,236 in 1861 to 2,729 in 1901. Yule (1920) concludes that "the main factor in the fall of the birth-rate has been a decrease in the fertility of married women; this fall has been proceeding at an accelerating speed."

The decline in the birth rate (so far as England and Wales are concerned) is not appreciably greater in the towns than in the rural districts. It is, however, especially marked in places inhabited by the servant-keeping class (Webb, 1907). It appears to be greatest in those sections of the population which give evidence of the exercise of thrift and foresight, for Heron (1906) has shown that the more cultured, prosperous, and healthy classes are producing fewer children than those belonging to a lower social status. Marriage fertility is on the whole graduated continuously from a very low figure for the upper and professional classes to a very much higher figure for unskilled labour (Carr-Saunders, 1922; Pearl, 1937b). As Webb (1907) has said:

"Modern civilisation is faced by two awkward facts: the production of children is rapidly declining and this decline is not uniform, but characteristic of the more prudent, foreseeing and restrained members of the community. . . . The most valuable of the year's crops, as it is the most costly, is not the wheat harvest or the lambing, but the year's quota of adolescent young men and women enlisted in the productive service of the community; and the due proportion and best possible care of this particular product are of far greater consequence to the nation, than any other of its occupations." (*See also* Whetham, 1909; Inge, 1920.)

It should here be emphasised that quality is far more important than quantity in child production, and that there are many powerful arguments to support the conclusion that somewhat smaller populations among all the nations of mankind may conduce to a general improvement in economic conditions. Moreover, a race for populations among the nations must be as harmful as a competition is in armaments.

The occurrence of multiple births has little influence on the birth rate; the birth of twins occurs once in about 80 deliveries. Triplets, quadruplets and quintuplets, and even higher figures, are occasionally observed; they are very uncommon and the rarity is progressive with the number. Veit's (1855) statistics for births in Prussia showed that twins were produced once in 89 cases, triplets once in 7,910 cases and quadruplets once in 371,125 cases. Greulich (1930), in a more recent estimate, finds that while twins occur once in 85 confinements, triplets occur once in 7,629 and quadruplets once in 670,734 cases of labour. There is some evidence that multiple pregnancy in women is commoner in cold than in warm countries (Williams, 1904; Greulich, 1930), and is less frequent in conceptions occurring during April-June and December, than at other times of the year (Edwards, 1938). The normal or ordinary rule in women is to bear one child at a time; and the next most frequent condition is temporary sterility (Duncan, 1866).

Duncan (1866) has shown that the fertility of wives increases gradually from the commencement of the child-bearing period of life until about the age of thirty

and then it gradually declines. Fertility is much greater before the climax (30 years) is reached than after it has passed; this refers, however, to the actual productiveness and not to the potential fertility. Charles (1934), however, gives data concerning births in Sweden which show that the actual fertility of wives decreases from 518 births per thousand at 15-19 years old to 312 at 30-34 years and to 20 at 45-49 years old.

There is, according to Pearl (1925, 1936a), a definite correlation between fertility and the frequency of coitus; the latter he found averages 12.6 times per month at 20-29 years old, whereas the rate drops to 8.2 at 50-59 and 5.3 at 60-69 years old. The later age at which marriage occurs in the upper as compared with the lower classes of society, reflecting as it does their desire for greater

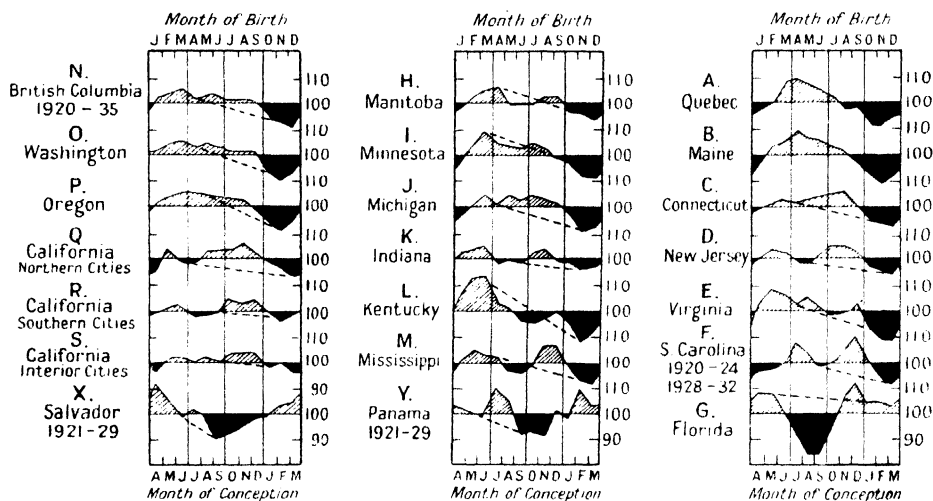


Fig. 21. 21.—The seasonal distribution of births in the United States and Canada (1926-32) and Central America (1921-29) showing the changes which occur in different latitudes. (From Huntingdon, 1938.)

Individuation, therefore tends to lower their fertility. The season of the year has a marked effect on the birth rate (*see* Fig. 21. 21); for the North Temperate Zone the basic annual rhythm reaches a peak for conceptions in May June, corresponding to births in February March (Huntingdon, 1938). For the southern hemisphere the curves are reversed; Huntingdon attributes these changes to optimum temperature conditions (62° F.) for reproductive activity; higher temperatures than this having a greater effect in depressing the rate than lower temperatures. It will be noticed that his curves shown in Fig. 21. 21 are very similar to those given for light effects on egg production in fowls shown in Fig. 21. 3.

There is no direct evidence that the decline in the birth rate that has taken place during the course of time in civilised countries is due to an increase of sterility, for congenital unavoidable sterility in either sex is comparatively rare. The inference has been drawn, therefore, that the decline is principally the result

of deliberate volition in the regulation of the married state. Evidence that this inference is correct is provided by the Fabian Society, whose report indicates that the practice of limitation prevails with at least one-half if not three-quarters of all married people of Great Britain. Statistics collected from other countries point in a similar direction. This is noticeably the case for New South Wales, Victoria and New Zealand for English-speaking peoples, and for France among Continental nations.

On the other hand, under conditions where the standard of living has not been increasing so fast (for example, among the Kaffirs), or where religious beliefs have been stronger than the urge for social improvement (for example among the Boers and the French Canadians) the birth rate has not fallen to anything like the same extent. There are, however, many indications that the decline in the birth rate (cf. Pearl — see above) is not due entirely, or even mainly, to the spread of the use of artificial modern methods of contraception (for details of which see Stopes, 1924 ; Dickinson and Bryant, 1932 ; Voge, 1933 ; Baker, 1935). Yule (1920), for example, has shown that in some countries (e.g. Belgium before 1875) the fall in the birth rate began at a time when these were not practised, and both he and Pell (1921) conclude that the recent decrease has not been effected mainly or solely by such a cause.

The wastage due to miscarriage is, however, considerable (*see* p. 703), but it is difficult to estimate, for natural abortions occurring at an early stage are frequently unrecognised as such, and as in most civilised countries induced abortion is a criminal offence no records of them are available. Natural abortions which can be recognised, mostly after 4 months, occur at various rates according to circumstances and tend to be greater in higher social levels of society. Pearl (1936b), for example, found it to be as low as 13 per 100 pregnancies in fertile negro women, whereas Dickinson and Beam (1931) found it as high as 30 per 100 in white women of high social status. In the incidence of still-births there is a marked difference according to the age of the mother ; from being 3·2 per 100 live births at 20-24 years old it rises to 4·9 at 35-39 years old and 8·7 at 45-49 years old (Pearl, 1936b). In very young mothers, too, the incidence is high.

One consoling factor in the general conclusion that improvements in the standards of living and in Individuation, make for a decline in the birth rate, is that under these conditions the survival rate of the children born is much higher. For example, child mortality (per 1,000 births) up to the age of one year has fallen from 151 in 1890 to 110 in 1915 and the death rate (per 1,000 population) from 18·9 to 14·2 during the same period in England and Wales (Charles, 1934).

We may conclude that under those conditions where the family income is improved by additions to the family, such as in the comparatively uncivilised countries where children add to the labour available to support life, and in the lower classes of civilised communities where the family live at home and become wage earners at an early age, a high birth rate will be encouraged. On the other hand, a high birth rate is discouraged under the conditions of highly civilised life where children detract from the family income owing to costs of education, etc. This is more especially so in the upper and professional classes where the children remain dependent on the family income for a long time, thereby not only deterring the parents from large families but also postponing the advent of their own reproductive life beyond that which is the most fertile period.

Against such biological laws of Individuation and Genesis, man-made laws and regulations concerning contraception and prostitution (the outcome and not the causes of a low birth rate) are of little avail. It is not until our statesmen can rearrange our order of life so as to harmonise with these biological concepts that birth rates may again be expected to increase in the civilised countries of the world.

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CHAPTER 22

THE FACTORS WHICH DETERMINE SEX

By F. A. E. CREW

I. THE NATURE OF SEXUALITY AND OF SEX-DIMORPHISM

Reproduction is either asexual or sexual. In asexual reproduction, in the unicellular Protista, for example, division of the nucleus entails division of the whole organism so that mitosis becomes a reproductive act. In multicellular organisms mitosis leads to increase in cell number and is a concomitant of growth. Under appropriate conditions this, like reproduction in the Protista, can continue indefinitely. In the higher plants, in which vegetative propagation can proceed without limit, reproduction is thus asexual. Reproduction by mitosis is widespread and is associated with great uniformity of characterisation within the species.

Sexual reproduction is that form of propagation in which meiosis (a modification of the mitotic process) and fertilisation are co-ordinated in the life cycle. Its function is that of providing a mechanism for the recombination of genetic differences. It is known in all groups of organisms in which there is an organised nucleus.

Before there can be sexual reproduction there must be sexuality, which in higher organisms implies sexual differentiation, and so this book, which deals with sexual reproduction, would be incomplete unless some reference were made to the problem of the origin and causes of the differences that distinguish the sexes, the male from the female. There are not only male-type and female-type individuals, zygotes; there are male-type and female-type gametes (spermatozoon and ovum) also, so that inquiries into the causes of sexual differentiation must consider both zygotic and gametic dimorphism.

A male, essentially, is an individual which produces male-type gametes (and is therefore equipped for their production), a female one which elaborates female-type gametes. In addition to this primary qualification, a male may possess many other attributes, morphological, physiological and psychological, which serve to distinguish him from a female, but these vary so greatly in their mode and degree of expression from species to species as to be unreliable as measures of maleness. But the one invariable property of the male is that of producing male-type gametes, which typically are small, active, motile and lean as contrasted with the typical female gamete, which is very much larger, has a larger nucleus and is heavily charged with nutritive material.

The morphological and physiological differences between male-type and female-type gametes are indications that the two kinds have their origins in gonads of different structure and function; the spermatozoa are elaborated in a testis, the ova in an ovary. The reasons for the differentiation of the gametes must

therefore be sought in the causes of gonadic differentiation. Since usually the testis is the peculiar property of the male and the ovary that of the female, it follows that the exact relation of gonadic type and sexual type of the zygote must be investigated. Is an individual a male because he is equipped with testes, or does he possess testes because he is a male? How and when is the sex of the individual determined?

In older days inquiries concerning the causes of the sexual differentiation of the zygote were always dominated by the preconceived notion that in all cases the newly fertilised egg was sexually undecided and neutral, and that conditions external to itself determined which of two alternative modes of sexual differentiation—towards maleness or towards femaleness—the developing individual should pursue. But recent developments in physiology, genetics and cytology have conclusively demonstrated the inadequacy of this view.

Physiologists have clearly shown that by the appropriate manipulation of the animal's functionings, the differentiating and, in certain forms though by no means all, even the differentiated individual can be made to assume, in whole or in part, the sexual characterisation normally expressed only by the opposite sex; a male can be induced to assume the female characterisation, a female can be partially or wholly transformed into a male; so that the physiologist does not regard the sexual characterisation of the individual as a fixed and final state, for he has shown that it is mutable and controllable. He looks upon maleness and femaleness as the end-results of differences in the balance between two opposing forces, a balance which, in some forms of living things, is apparently stable and permanent, whereas in others it is impermanent and more or less easily disturbed. Dioecious forms (in which maleness and femaleness are separated) have been transformed experimentally into monoecious or hermaphroditic forms in which one and the same individual is at one time a male and at another a female (hermaphroditic in time) or else at one and the same time is equipped with both male and female types of sexual and reproductive apparatus (hermaphroditic in space). No male is wholly male, no female solely female, for the physiologist has disclosed in both the presence of those endocrine agents which, given opportunity, can control bodily differentiation and lead to the development of each of the two types of sexual architecture. Every individual elaborates both male-differentiating and female-differentiating substances, but in the normal male the former are in excess (either in quantity or potency) during that phase of development when the sexual characters are assumed and continue to be expressed, whereas in the normal female, during this same period, the female-differentiating substances are predominant. If this balance is disturbed, then the sexual characterisation of the individual can become altered.

These would seem to be the conclusions, stated in the simplest terms, to which the physiologists have arrived. They relate to the processes of differentiation, to the physiological properties of differential cells, tissues and organs, and to the reactions of the body to the stimuli these provide. They are, however, the same conclusions which the geneticists and cytologists have reached, though by a totally different route. Among these it is now generally accepted that, in a very large number of living things, the zygote, in virtue of its initial genetic constitution, established at the time of fertilisation, is predisposed towards one or other of the two alternative modes of sexual differentiation; the zygote being from its beginning

a *genetic* male or else a *genetic* female ; that in many forms this predisposition is all-powerful, amounting in fact to predestination, whilst in others it is but slight and more or less easily overwhelmed by extra-zygotic forces, so that it may come to pass that the ultimate sexual characterisation (the sexual phaenotype) is not in harmony with the genetic constitution (the genotype). The geneticist, like the physiologist, thinks of maleness and femaleness as the results of differences in the balance between two conflicting forces, the elaborated chemical products not of cells and tissues but of the genes themselves. There are genes which are male-determining (M) and genes that are female-determining (F) ; both kinds are present in the genotype of each and every zygote. In the male the male-determining genes are more numerous or more powerful, $M > F$, whereas in the female, $F > M$; these initial genetic differences are the causes of all subsequent sexual differentiation. A male, because he is a " genetic " male, becomes a functional male equipped with testes, and therefore elaborating male-type gametes. A female, because she is a " genetic " female initially, becomes a " phaenotypic " female, possessing ovaries and therefore elaborating female-type gametes. The gametes produced by such males and females may be genetically identical or genetically dissimilar, but they are morphologically and physiologically markedly different.

These conclusions are based upon the fruits of decades of eager genetical and cytological investigation which has disclosed a sex-determining mechanism, exceedingly simple in design and most efficient in action. It can be regarded as the highly finished product of a long and adventurous evolutionary process, tested and of proven worth. Before it could come into being much had to happen. The organised nucleus had to appear, and the chromosomes had to pursue their own evolutionary history, breaking and reuniting until a particular equilibrium had become established. Meiosis had to become perfected, and much mutation had to take place. This being so, it need surprise no one that, in a great many forms of life, a mechanism of such delicate precision appears to be lacking. In these the decision as to whether a particular zygote shall develop into a male or else into a female often seems to be made by circumstances completely external to itself ; environmental, extra-zygotic factors alone seem to be concerned. That this is so may be due either to the absence of an intra-zygotic sex-determining mechanism or to its imperfection or impotency, this being such as to render the mechanism liable to be more or less easily overridden by the impress of environmental forces. It is in fact, at the present time, most difficult, if not impossible in many instances, to decide which of these two explanations is the more probable. However, it is convenient and reasonable, on general grounds, to adopt the view that, in some cases, sex is determined solely by genetic factors, in others by environmental factors alone, and in still others by a combination of genetic and environmental factors.

Genetic.—Genetic, constitutional, intra-zygotic factors determine the sex of the individual. Environmental factors play either no part or else so slight a part that their separate action cannot be recognised. Example : *Drosophila melanogaster*.

Environmental.—Environmental (extra-zygotic) factors determine the sex of the individual. Genetic factors play no part or else so slight a part that their separate action cannot be recognised. Example : *Dinophilus apatris*.

Genetic plus environmental.—Genetic factors predispose the zygote towards either male-type or female-type differentiation, but for such differentiation to take place, the presence of certain special environmental factors is required. All the visible qualities of any organism are, of course, the result of the reaction of its constitution with its environment ; genetic characters can only become expressed, and genes can only act, with the permission or with the encouragement of environmental factors. Every attribute of the living organism is a product of the interplay of intra- and extra-zygotic factors. But the environmental factors operating in this genetic-environmental form of sex-determination are not thought of as being vague and general ; they are quite definite and specific, being such as, for example, affect the oxidation rate or influence the rate or direction of some definite developmental process.

It has to be confessed that, though it may be possible for purposes of discussion to distinguish between the environmental and the genetic-environmental types of sex-determination, in practice it is almost impossible to do so, and as a result there is much argument concerning doubtful instances. As an example showing the difficulties that are encountered in the analysis of a case of this kind, *Bonellia viridis* is usually cited.

II. THE GENETIC SEX-DETERMINING MECHANISM

In many forms this mechanism can be displayed in all its precise perfection. It was first envisaged after it had been observed that, with certain exceptions, the process of fertilisation resulted in the production of two kinds of individuals, males and females, in approximately equal numbers. The orderliness of this production of the two sexes, as a consequence of the mating of male and female, was regarded as an indication that sex-determination must be controlled by some relatively simple intra-zygotic mechanism. The character-constellations, maleness and femaleness, appeared and reappeared in successive generations in a fashion that, when once the Mendelian scheme of organic inheritance was recognised, suggested strongly that they were themselves a pair of Mendelian characters, and that one sex was a constitutional heterozygote ($AA \times Aa = AA$ and Aa in equal numbers). This hypothesis was quickly put to the test of experiment. Doncaster (1916), working with the currant moth *Abraxas*, Durham and Marryat (1908) with the canary, Bateson and Punnett (1908) with the fowl, were prominent among those who first used animal material in investigations of this kind, and furnished genetic evidence which conclusively demonstrated the existence of an intra-zygotic sex-determining mechanism. They discovered sex-linkage, a mode of hereditary transmission which, for its interpretation, demanded that a sex-determining mechanism of the $AA : Aa$ type must exist and that the hereditary factors corresponding to the characters they were studying must be attached in some way to the elements of this mechanism.

During the last twenty-five years a very considerable number of these sex-linked characters have been recognised in a variety of forms which all belong to one or other of two groups—to that in which, according to the genetic evidence, the female is of the Aa constitution, the male AA , or else to that in which it is the male that is Aa and the female AA . According to Mendelian theory, the Aa

zygote must produce A and a gametes in equal numbers as the result of segregation ; such an individual, producing two kinds of gametes differing one from the other in respect of their factorial constitution, is a sex-heterozygote, and is therefore heterogametic ; the AA individual is a homozygote and homogametic.

In the history of the science of genetics an event of outstanding importance was the recognition of the fact that the chromosomes of the nucleus and the mechanism concerned with their distribution from cell generation to cell generation furnished an entirely satisfactory mechanism for the distribution of hereditary characters as postulated by Mendel (1865) and indeed satisfied every demand made by this theory in respect of gametic purity, segregation, free assortment and recombination, linkage and sex-linkage. Cytological discoveries revealed that fertilisation was, essentially, the fusion of the nuclei of two morphologically dissimilar gametes, each with the same number of chromosomes, so that the zygote resulting from their union came to possess a double (diploid) number. During gametogenesis the compensating process of meiosis, during which two nuclear divisions followed rapidly one upon another while the chromosomes themselves divided only once, gave rise to gametes each of which contained the halved or haploid number of chromosomes. In cases in which the chromosome number was small and the chromosomes themselves heteromorphic, differing among themselves in size and shape, it was seen that in the zygote the chromosomes were present in the form of pairs, whereas in the gamete there was but one representative of each of these pairs of homologous chromosomes. Furthermore, the somewhat premature, but nevertheless brilliant, identification of sex differences in respect of chromosomes by McClung (1901) was soon corrected and confirmed, for Stevens (1905) and Wilson (1905), examining several species of Hemiptera, showed beyond all doubt that in the males of these forms there was one pair of chromosomes which consisted of mates of unequal size, and that the members of this pair separated and passed to opposite poles in one of the two meiotic divisions. Stevens then proceeded to show that the diploid number of chromosomes was the same in both male and female, and that in the female there was no pair consisting of unequal mates. So cytology provided that which genetics was demanding, a segregation of dissimilar elements of a sex-determining mechanism which could carry the hereditary factors corresponding to the sex-linked characters.

The chromosomes of that pair in respect of which the sexes differ are known as the sex-chromosomes (or as the hetero-chromosomes), the rest, in respect of which male and female are alike, the autosomes. The two sex-chromosomes of the one sex and that member of the same pair in the opposite which is like these, are the X-chromosomes ; the unequal mate of the X is known as the Y-chromosome. The XY individual possesses two sets of autosomes and a pair of sex-chromosomes, XY. The gametes elaborated by such an individual will be of two kinds, one containing a single set of autosomes together with an X, the other a set of autosomes and a Y. The XX individual possesses two sets of autosomes and a pair of sex-chromosomes, XX. The gametes elaborated will be all of a kind in respect of chromosome content, having one set of autosomes and an X. In all animal species examined, save in the Lepidoptera, Trichoptera, birds and some fishes the heterogametic (XY) sex has been found to be the male. In the Reptiles sex-chromosomes cannot be identified cytologically in either sex, so that in the absence of genetical evidence it is still uncertain which is the heterogametic sex.

(Matthey, 1943.) As would be expected, it is not always possible to identify the sex-chromosomes by reference to size differences between the X and the Y. In some forms the distinction is easy for the reason that the Y is lacking altogether (most Orthoptera, many Hemiptera, Coleoptera and Nematoda), so that the heterogametic sex is XO and not XY. However, the cytologist has devised other methods for their recognition. Commonly these chromosomes differ from the autosomes in respect of behaviour; in mitosis and meiosis they may undergo a precocious condensation, remain condensed for a longer period of time, or they may occupy special positions on the spindle. In some forms (e.g. Culicidae, Chironomidae, Pisces, Amphibia) the evidence for their existence is purely genetic (Darlington, 1937; Oguma and Makino, 1937; White, 1945).

This XY (or XO) : XX mechanism is an exceedingly simple and most satisfactory device for the production of two sexes in equal numbers.

Parents (gametogenesis)	Male Heterogamety			Female Heterogamety		
	♂ XY	×	♀ XX	♀ XX	×	♂ XY
Gametes (fertilisation)	X 50%	:	Y 50%	X	X : Y	
Offspring	XX ♀♀ 50%		XY ♂♂ 50%	XX ♂♂ 50%		XY ♀♀ 50%

Moreover, if there are hereditary factors, genes, resident in the X-chromosomes and not in the Y then their mode of transmission must necessarily differ from that of genes resident in the autosomes. Of each pair of autosomes in the nucleus of the zygote, one member is derived from the father, the other from the mother. The same is true of the sex-chromosome pair of the XX individual, but the XY or XO zygote gets its single X from one parent, the parent of the sex opposite to its own, and with this X pass the genes resident therein. A character is transmitted in a sex-linked manner because the gene corresponding to it is resident in the X-chromosome. A few examples will illustrate the soundness of this contention.

The Inheritance of Haemophilia in Man

X = the X-chromosome : h = the gene for the sex-linked recessive character haemophilia. H = the dominant normal allelomorph of this recessive gene. Brackets include an X-chromosome and the gene it carries. It is accepted that on every X-chromosome there is either the gene h or else its allele H. The male has a single X, this can be either (hX) or (HX), and since there is no gene in the Y to affect the expression of these genes in the X, a male is either haemophilic (hX)Y or else normal (HX)Y. A female, on the other hand, having two Xs can be (HX)(HX) normal, (HX)(hX), a "carrier," heterozygous, or else (hX)(hX), haemophilic.

(a) haemophilic ♂	(hX)Y	×	(HX)(HX)	normal ♀
	(hX) : Y	::	(HX)	gametes
	(HX)(hX)	:	(HX)Y	
“carrier” daughter		:	normal son.	
(b) normal ♂	(HX)Y	×	(HX)(hX)	carrier ♀
	(HX) : Y	::	(HX) : (hX)	gametes
	(HX)(HX) : (HX)(hX)	:	(HX)Y : (hX)Y	
normal daughter	carrier daughter		normal son	haemophilic son

The sons receive their Y-chromosome from their father ; this can be disregarded. They receive their single X from their carrier mother : she has two kinds to offer, (HX) and (hX) respectively. The son that receives an (hX) chromosome will inevitably be haemophilic. In this way the facts concerning the transmission of haemophilia are accommodated easily. It is a sex-linked recessive character and the human male must have an XY sex-chromosome constitution. Cytological investigation has shown that in the human zygote there are twenty-four pairs of chromosomes, that the male is XY and the female XX (Painter, 1923 ; Evans and Swezy, 1929 ; Koller, 1937).

The Inheritance of Silver Plumage Colour in the Fowl

S = the dominant gene for silver ; s = the allelomorphous recessive gene for “gold” (not silver).

gold ♂	(sX)(sX)	×	(SX)Y	silver ♀
	(sX)	::	(SX) : Y	gametes
	(SX)(sX)	:	(sX)Y	
heterozygous silver sons			gold daughters.	

A male bird, to be a male, according to the argument, must have two Xs. Of these he must receive one from each parent. One of them must therefore be carrying the dominant silver gene since the mother has only this to offer. All the sons must therefore necessarily be silver. A female bird, to be a female, must be XY. The Y she must receive from her mother, the X from her father. He has but one kind of X (sX), and so the female offspring of this mating will all be golds.

It is a fact that the mating gold cock × silver hens gives gold daughters and silver sons, daughters that exhibit the character that their father displayed, whilst sons “take after” their mother (criss-cross inheritance). There is but one explanation of this, gold and silver are sex-linked characters, gold is the recessive allelomorph of silver, their genes must be X-borne, and the male must be XX and the female XY or XO. Cytological studies have shown that in birds the male is XX (White, 1932 ; Crew, 1933 ; Miller, 1938). It is not yet finally established whether in the fowl the female, as in other birds, is XY or XO (Painter and Cole, 1943).

Non-disjunction of the Sex-chromosomes

The rôle of the sex-chromosomes in sex-determination is even more clearly illustrated by reference to Bridges' (1916) work on non-disjunction. In *Drosophila melanogaster* the mutant recessive eye colour white is a typical sex-linked character. w = the gene for white eye colour ; W = its dominant wild-type allele giving red colour.

red-eyed ♂	(WX)Y	×	(wX)(wX)	white-eyed ♀
	(WX) : Y	::	(wX)	gametes
	(WX)(wX)	:	(wX)Y	
	red-eyed		white-eyed	
	daughters		sons	

But though this is the expected and usual result of such a mating, not infrequently exceptional white-eyed daughters and red-eyed sons appear among the progeny. The reason for this was shown to be non-disjunction of the sex-chromosomes, the white-eyed mother being XXY instead of the normal XX. She has her origin in the fertilisation by a Y-chromosome bearing sperm of an egg into which both X-chromosomes had passed, through failure on their part to disjoin during gametogenesis. Normally they separate, one going into the polar body and the other remaining in the ovum. Exceptionally, both pass together into the polar body, or both remain in the ovum. Such exceptional eggs may be fertilised by X- and by Y-bearing spermatozoa.

ovum XX	— X sperm	= XXX	a triplo-X female ; “super ”-female with exaggerated female characteristics
„ O — X	„ — XO		a normal-looking but infertile male
„ XX — Y	„ = XXY		a female apparently quite normal
„ O — Y	„ = OY		non-viable.

If the white-eyed female used in a mating with a red-eyed male happens to be (wX)(wX)Y in constitution, she will provide four kinds of eggs instead of one, in respect of sex-chromosome content. There are available for fertilisation two kinds of spermatozoa, (WX) and Y, respectively.

Spermatozoa	Ova	Zygote
	(wX)(wX) —	(WX)(wX)(wX) triplo-X red-eyed ♀
	Y —	(WX)Y exceptional red-eyed ♂
(WX) —	(wX) —	(WX)(wX) red-eyed ♀
	(wX)Y —	(WX)(wX)Y red-eyed ♀
	(wX)(wX) —	(wX)(wX)(wX) exceptional white-eyed ♀
	Y —	YY non-viable
Y —	(wX) —	(wX)Y white-eyed ♂
	(wX)Y —	(wX)YY white-eyed ♂

The exceptional red-eyed sons are red-eyed because they received their single X-chromosome from their father (WX) and their Y from their mother. The exceptional white-eyed daughters are white-eyed because they received both their X-chromosomes from their mother (who exhibited the recessive white-eye

character). These genetic findings, confirmed by cytological examination, showed that one X is invariably associated with maleness, two Xs with femaleness, and that the Y is unimportant save that it is necessary for fertility (Safir, 1920) or, as Stern and Hadorn (1938) and Neuhaus (1939) have shown, for the mobility of the sperm. That this non-disjunction explanation was correct was proved by a variety of genetical tests and by cytological observations, all of which went to show that the X-chromosomes were the actual determiners of sex and were not merely indices.

Gynandromorphism in Drosophila

Another, and equally fruitful, source of evidence concerning the sex-determining mechanism is the gynandromorph of *Drosophila melanogaster*. A gynandromorph is a sex-mosaic in space, an individual exhibiting male and female characterisations in different regions of its body. Morgan and Bridges (1919) were able to show that, since in these flies the abnormality involves only the sex-linked characters, male and female areas in these sex mosaics must differ from each other in respect of their X-chromosome content. A fly, for example, has one lateral half of its body with the male characterisation and with a white eye, while the other half is female and red-eyed. It is reasonable to assume that in this case fertilisation yielded a zygote which was a heterozygous red-eyed female (WX)(wX). Maldistribution of the chromosomes at the first division of the fertilised egg resulted in the exclusion of an X-chromosome, in this case a (WX), from the nucleus of one of the resulting daughter-cells. These thus became (WX)(wX) and (wX) respectively. If then from each of these daughter-cells one lateral half of the body is derived, those cells concerned with the developing eye on one side of the body all possess the (wX) chromosome, and so the eye colour on this side is white. The eye of the other side is red because the genetic constitution of the tissues on this side is (WX)(wX), and red-eye is dominant. Furthermore, the tissues of the one side possessing but a single X would develop according to the male plan, whilst those of the other, being XX, would pursue a normal female-type development. If this is so, then it follows that, in *Drosophila* at least, the sexual destiny of an individual or of any part thereof is decided once and for all by its genetic constitution. But not all gynandromorphs are examples of the effects of chromosome elimination. A few have been encountered in *Drosophila* as well as in other forms which are much more satisfactorily explained by an appeal to the double fertilisation hypothesis advanced by Doncaster (1916) in connection with the gynandromorphs of *Abraxas*.

The genetic and cytological evidence so far reviewed points directly to the conclusion that the significant and fundamental difference between male and female is in respect of the number of X-chromosomes. This, though true, is not the whole truth. The work of Bridges (1922, 1925) on the progenies of triploid females of *Drosophila*, demonstrated that autosomal genes were also and equally concerned. Among these progenies were individuals of varied sexual characterisation ranging from super-males through males, male intersexes, female intersexes, females, to super-females. The succession in this series was shown cytologically to be constantly related to the proportions in which the autosomes and the X-chromosomes were present in the individual. Normally there are four

pairs of chromosomes, pair number one being the sex-chromosomes consisting of two rod-like Xs or of an X and a hooked Y, pairs number two and three consisting of long boomerang-shaped autosomes, whilst the fourth pair are minute and dot-like. If $X = 1$ X-chromosome and $A = 1$ haploid set of autosomes, the series can be presented as follows :

				4X : 4A	
	1X : 2A			3X : 3A	
1X : 3A	1XY : 2A	2X : 3A-IV	2X : 3A	2X : 2A	3X : 2A
1XY : 3A	1XYY : 2A			2XY : 2A	
				2XYY : 2A	
				1X : 1A	
super-males	males	male-type intersex	female-type intersex	females	super- females

(In the male-type intersex the second and third chromosomes are present in triplicate, but of the fourth there are but two.)

Of the above types only the complete haploid $1X : 1A$ was lacking. Such an individual has, in fact, never been found in *Drosophila* or in any kindred species. Nevertheless the idea that this particular chromosome combination was female-determining and not male-determining was confirmed by Bridges himself when he encountered two mosaic flies which were demonstrably haploid in respect of certain areas of their bodies ; these areas exhibited a female characterisation. Later, Crew and Lamy (1938) described some forty of these haplo-diploid mosaics in a strain of *Drosophila pseudo-obscura*, the haploid area in every case exhibiting femaleness equally with the normal diploid ($2x : 2A$) area and in one case including the ovary, which by genetic tests was shown to have elaborated normal gametes.

There is thus no escape from the conclusion that sex-determination is the result of a quantitative balance between the X-chromosomes and the autosomes and that this X is not the determiner of sex but is a differential. Genes concerned with sex-determination are scattered throughout the chromosomes. In a general way they may be classified as male-determining and female-determining. In the X and on the small IV chromosome (which probably arose as a detached fragment of the X), female-determining genes have become congregated whilst on the rest the male-determining genes have become collected. The sex of the individual is decided by the genic balance which rests upon the ratio of the number of X-chromosomes and the number of autosomes.

If this is so, then the female of *Drosophila*, having two X-chromosomes, has two sets of female-determining genes in association with two sets of male-determining genes on the autosomes. She can be symbolised, therefore as $2M : 2F$, and it may be assumed that $2M < 2F$. On the other hand, the male, having but one X, becomes $2M : 1F$, and $2M > 1F$. So once again we arrive at the conclusion that, in each zygote, from the beginning, there are two tendencies working in opposite directions, towards maleness and towards femaleness, and that the stronger gains control.

The work of Dobzhansky and Schultz (1931) has shown that by adding X-chromosome fragments of different sizes to the nuclear content of *Drosophila* intersexes can be obtained. By these means it becomes possible to alter the grade

of intersexuality in varying degrees, but always in the female direction, according to the nature of the fragment thus added. The conclusions reached by these authors was that "the sex-determining rôle of the X-chromosome of *Drosophila* is due to a co-operative effect of numerous female modifiers located in all regions of the chromosome." More recent work by Patterson and his collaborators (1940) has completely confirmed these conclusions.

The X and Y chromosomes are to be looked upon as modified autosomes. Their sex-determining function depends upon the fact that the X contains a region (or regions) which is not present in the Y and which bears a number of sex-determining genes (female-determining in most groups, male-determining in a few such as Lepidoptera, Trichoptera and birds). The Y may also include a special region (or regions) not present in the X. The evolutionary history of the sex-chromosomes themselves may be regarded as a progressive increase in size of these differential regions at the expense of the homologous regions so that the X and the Y, originally alike, have come to be more and more different.

Another species of *Drosophila*, *D. miranda*, possesses a modification of this XY mechanism which has also been encountered in other groups of insects. The female has two pairs of sex-chromosomes, X^1 and X^2 , whereas the male is hemizygous for both and in addition carries a Y (Dobzhansky, 1935). The two X-chromosomes of the male must go, at spermatogenesis, into the same spermatogonium whilst the Y passes into the other. The mechanism which is responsible for this event is as yet not entirely clear (MacKnight and Cooper, 1944).

A few species are known in which there are multiple Xs and no Y and in a very few animals there is a multiple Y. Such multiplicity of sex-chromosomes is relatively common in Nematoda, Reduviidae, Mantoidea and spiders. The case of the Praying Mantis is the clearest of all. In many species the males are X_1X_2Y , the females $X_1X_1X_2X_2$ (White, 1941). There are two main ways in which such multiple mechanisms may have arisen, (a) by the inclusion of autosomes or parts of autosomes in the sex-determining mechanism as a result of translocation, and (b) by the reduplication of parts of the original sex-chromosomes. Most instances seem to have arisen by the second method (White, 1940).

It doubtless will appear to many that *Drosophila* has figured too prominently so far in this survey. Yet it has claimed no more space than it can command. To this small creature we owe most of our knowledge of matters genetical; its special value as a point of departure in this study lies in the fact that it is ideal genetical animal material and therefore the material with which most work has been done, and concerning which most is known. It has been used, of course, for genetical and not for physiological purposes, and this is one reason why the geneticist, referring to *Drosophila* for his facts and their interpretation, commonly finds himself in disagreement with the interpretation of those who have used other experimental material of unknown genetical and cytological constitution in physiological rather than genetical investigations. But such disagreement is mainly one of interpretation and will doubtless disappear when the developmental physiology of *Drosophila* has been extended and the cyto-genetics of the physiologist's material develops.

After *Drosophila*, the gypsy moth *Lymantria dispar*, particularly in the hands of Goldschmidt (1934) has contributed most to our knowledge of the factors

concerned in the determination of sex. This moth is widespread in its distribution and of it there are a number of geographical races. It has long been known that a common result of the hybridisation of these races was the appearance of a number of intersexual forms among the progeny. Goldschmidt carried out hybridisation of this kind for twenty years and more and found that it was possible, by making the appropriate matings, to produce definite grades of this intersexual condition. He found that there was a time seriation of events in the development of the sex characters and that the grade of intersexuality was determined by the relative number of these structures that were male-type and female-type respectively. It was seen that the last structure of the sex-equipment to be differentiated in the life history of the individual was the structure most commonly involved in the intersexual condition, and that a greater number of these structures became affected with each increasing grade of intersexuality. The results Goldschmidt obtained by crossing the different races permitted him to classify these races as "strong" or "weak" according to the "valency" or "potency" of their sex-determining factors. There were "strong" and "weak" male and female-determining factors. The male-determining factors were resident in the X-chromosome, the female-determining in the cytoplasm, and therefore restricted to the female line. The crossing of different races brought together male-determining and female-determining factors of different valencies so that M, the male-determining factor complex, might be greater than, equal to, or less than F.

To illustrate Goldschmidt's hypothesis in simple terms is somewhat difficult. Perhaps the easiest way is as follows:—

M represents the gene complex yielding maleness and resident in an X chromosome.

m represents the absence of an X chromosome (or the presence of a Y chromosome).

F is the factor complex borne in the cytoplasm of the egg and yielding femaleness.

The relative valency of an M or F is indicated by a numeral, $M_1M_2M_3M_4$, $F_1F_2F_3F_4$, etc.

Since a male has two Xs and therefore two Ms, the latter can be of different valencies.

$$\begin{array}{rcll}
 (1) & \begin{array}{l} \text{♂} \\ \text{Gametes} \\ \text{Offspring} \\ \text{male} \end{array} & \begin{array}{l} M_3M_3F_4 \\ M_3 \\ M_3M_3F_4 \\ XX \\ M > F \end{array} & \begin{array}{l} \times \\ M_3m F_4 : m F_4 \\ M_3m F_4 \\ XY \\ M < F \end{array} \begin{array}{l} \text{♀} \\ \text{female} \end{array}
 \end{array}$$

$$\begin{array}{rcll}
 (2) & \begin{array}{l} \text{♂} \\ \text{Gametes} \\ \text{Offspring} \\ \text{male} \end{array} & \begin{array}{l} M_2M_2F_3 \\ M_2 \\ M_2M_2F_3 \\ XX \\ M > F \end{array} & \begin{array}{l} \times \\ M_2m F_3 : m F_3 \\ M_2m F_3 \\ XY \\ M < F \end{array} \begin{array}{l} \text{♀} \\ \text{female} \end{array}
 \end{array}$$

(3) Now the cross between these two races :

♂	$M_3M_3F_4$	×	$M_2m F_3$	♀
Gametes	M_3		$M_2F_3 : m F_3$	
Offspring	$M_3M_2F_3$		$M_3m F_3$	
male	XX		XY	female
	$M > F$		$M = F$	and therefore intersexual

(4)

♂	$M_2M_2F_3$	×	$M_3m F_4$	♀
	M_2		$M_3F_4 : m F_4$	
F_1	$M_2M_3F_4$	×	$M_2m F_4$	
	$M_2 : M_3$		$M_2F_4 : m F_4$	
F_2	$M_2M_2F_4 : M_2m F_4$		$M_3M_2F_4 : M_3m F_4$	
	XX	XY	XX	XY
	$M = F$	$M < F$	$M > F$	$M < F$
an inter-		♀	♂	♀
sexual male.				

Sex-determination in *Lymantria*, as in *Drosophila*, is the result of genic balance. In the uneventful differentiation of the normal male, M is always effectively in excess of F, in the case of the normal female, F is always effectively in excess of M, but in the development of the intersex, the relation of M and F is disturbed, M (or F) overtakes and replaces F (or M) at some point during the period when the sex-equipment is becoming differentiated—the turning-point or switch-over. The effect of this genic situation is that, at a certain moment in development, the switch-over occurs and the control of the remaining events is shifted from the F to the M genes (or from the M to the F) and the time of occurrence of this event is the simple function of the relative degree of balance or imbalance between M and F (the Time Law of Intersexuality, Goldschmidt, 1938). M and F respectively are responsible for sex-differentiating reactions which proceed with a velocity proportional to the “strength,” “valency,” or “potency” of these genes; the “quicker” reaction controls the sexual differentiation and the two curves of the M and F reactions may have points of intersection if the “quantities” of M and F are not properly matched.

It will be seen that those who have learnt from *Drosophila* and from *Lymantria* respectively are in agreement in holding the view that sex is determined by a balance between male- and female-determining factors. But, whereas the *Drosophila* school is forced to think in terms of many factors qualitatively different but acting together in quantitative relation or ratio, the evidence furnished by *Lymantria* and as interpreted by Goldschmidt suggests that there are quantitative differences among the male-determining genes and among the female-determining. Each of these preferences has its own advantages and limitations. The two should be regarded as supplementary one to the other, and certainly not as contradictory. When these concepts can be expressed in biochemical terms genetics and physiology may strongly reinforce each other. An instance of such reinforcement is seen in the work of Moewus and Kuhn on *Chlamydomonas* and Kimball on *Euploides* presented in the review of Sonneborn (1942) on the unicellular alga *Chlamydomonas*.

In *Lymantria*, as in *Drosophila*, the sexual fate of the zygote is to a large extent fixed by its initial genotype. From the moment of fertilisation the individual is destined to become male, female or intersex. But in *Lymantria* it has been found possible, by controlling the temperature during development, to produce sexually abnormal forms within a pure race, and, in *Drosophila*, to shift in one direction or the other the degree of the intersexuality of the intersexes (Dobzhansky and Bridges, 1928). Goldschmidt assumes that, by altering the temperature, he changes the relative velocities of the male and female reactions. So that it seems that, in *Lymantria*, extraordinary extra-zygotic factors can modify sexual predestination, whereas in *Drosophila* this predestination is only to be found in the case of intersexes in which the balance is unusual.

There are actual genes which in their action yield intersexuality. The first of these was discovered by Sturtevant (1922) in the second chromosome of *D. simulans*. Its action is to transform the female into an intersex. Lebedeff (1938) found another in the third chromosome of *D. virilis* which, leaving the male unaffected, actually transforms the homozygous female into a sterile male. Later, he encountered other genes which modified the action of this gene, delaying the process of sex-transformation, and thus yielding hermaphrodites. More recent work, especially that of Newby (1942), who studied a gene which produces intersexes in *D. virilis*, has led to a reconsideration of Goldschmidt's Time Law of Intersexuality. It would now seem probable that the phenomena encountered in the *Lymantria* intersexes will be found to be interpretable in terms of the observed details of development that Newby has observed in *D. virilis*.

The production of hermaphrodites in this fashion is a matter of great interest. Apparently in *D. virilis*, though not in *D. melanogaster* and *Lymantria dispar*, the development of the individual can, at one and the same time, be guided by both male-differentiating and female-differentiating influences. Lebedeff is inclined to look upon the normal allele (IX^m) of this mutant intersex gene iX^m as a normal male-determiner, and the iX^m gene itself as being very "strong," so much so that it partially overrides the $2X:2A$ balance, turning females into sterile males. Sex, he thinks, is determined by the balance between a single gene for femaleness in the X-chromosome and a single gene for maleness, IX^m , in the third chromosome, the two being equally potent. A set of "suppressor" genes, which inhibit the activity of the IX^m gene, is responsible for the production of females. The XY mechanism he visualises as the controller of the suppressors which are specifically suppressors of maleness.

Though the instances of sex-determination by means of a genetic mechanism so far cited differ among themselves in respect of detail, they are all alike in that in them heterogamety exists in one sex. They all are in agreement with the observations of Boveri (1909) and Gulick (1911) on the nematode *Heterakis*, of Morrill (1910) on the maturation of Hemipteran eggs, and of Mulsow (1912) on the nematode *Ancyracanthus*. In this nematode the chromosomes remain separate in the sperm and can be counted. They are visible even in life. The primordial egg cells in the ovary contain 12 chromosomes and during synapsis six double elements are to be seen. The first maturation division removes six complete chromosomes from the egg into the first polar body whilst the second division splits each remaining chromosome longitudinally and separates the resulting halves. The mature egg therefore contains six chromosomes, one of

which is the X. In the primordial cells of the testes of the male there are eleven chromosomes, one of which is the unpaired X. During conjugation in the synapsis stage the X can be seen to have no partner and to remain aloof. In the first maturation division there are five bivalent chromosomes and the univalent X. When these chromosomes are distributed to the daughter cells the X passes undivided to one or to the other. This division yields two primary spermatocytes, one of which has five chromosomes, the other six. The second meiotic division produces four spermatids, two with five and two with six chromosomes. As the spermatids mature to become spermatozoa these chromosomes remain separate and visible.

In other forms the relation of the sex-chromosomes to sex-determination is not so clear. In the midge *Sciara*, for example, there would appear to be two kinds of females, male-producers and female-producers respectively, the two differing from each other in respect of their X-chromosome content. Apparently there are two kinds of X-chromosomes, the ordinary X and a peculiar X^1 . Male-producing females are, according to Metz (1925, 1938), XX, whilst female-producing females are X^1X . The eggs contain one X-chromosome, the spermatozoa two. Individuals of each sex start as fertilised ova with three X-chromosomes. The cytological type of the mother determines whether the egg, by eliminating both paternal Xs from the soma, will develop into a male, or alternatively, by eliminating one paternal X only, into a female. Both X^1XX and XXX eggs from an X^1X mother develop into females and all the XXX eggs from an XX mother develop into males. Thus *Sciara* appears to be a case of predetermination of sex through the cytoplasmatic constitution of the egg before maturation and fertilisation (Du Bois, 1932).

III. THE HAPLO-DIPLOID SEX-DETERMINING MECHANISM

We know that another type of chromosomal sex-determining mechanism, as simple and as efficient as the $1X : 2A : 2X : 2A$ device, is widespread. This is the haplo-diploid sex-determining mechanism such as is found in the Hymenoptera, Rotifera, in some Thysanoptera, Acarina and Hemiptera. In these the females are diploid, $2X : 2A$, whilst the males are haploid, $1X : 1A$. The evidence for this, especially in the case of the Thysanoptera and Rotifera, is chiefly from the results of experimental breeding, but in quite a number of instances the male has been shown to be haploid by cytological examination (cf. Darlington, 1937). In the bee, wasp and ant it is established that unfertilised eggs develop into males, fertilised ones into females. Nothing could be simpler than this as a method of producing males and females, but the ratio between X and A is the same in both male and female and so the application of the genic balance hypothesis is attended by considerable difficulty, especially as it is known that $1X : 1A$ tissue in *D. melanogaster* is female.

Whiting and his collaborators (1943), working with the parasitic wasp *Habrobracon*, have successfully attacked the problem. Exceptionally, diploid males are found among the progeny of a mated female, so that the $1X : 1A$ constitution cannot be the cause of maleness or the $2X : 2A$ the essential concomitant of femaleness in this wasp. These diploid males are produced far more frequently in inbred matings than in outcrosses. Whiting and his colleagues have brought

Habrobracon into line with *Drosophila*. There are in *Habrobracon* a number of factors which, when heterozygous, determine femaleness, when homozygous (as in exceptional diploid males) or hemizygous (as in normal parthenogenetic males) determine maleness (Whiting, 1943).

The cytological concomitants of parthenogenesis have been extensively studied in many groups of animals. Parthenogenesis is either diploid or haploid; in the first case the parthenogenetically produced offspring have the diploid number of chromosomes and in the second, the haploid. In diploid parthenogenesis the progeny may be either males or females (aphids, daphnids, gallflies), whereas in haploid parthenogenesis they are always males (rotifers, bees). In some cases of diploid parthenogenesis there is no meiosis, while in other instances a reduction to the haploid number occurs, the diploid chromosome number being restored, either by fusion of the second polar-body nucleus with the egg nucleus or by fusion of the cleavage nuclei in pairs (Seiler, 1923, 1943; Vandel, 1931; White, 1945). In a few instances parthogenetic species of animals are polyploid (Vandel, 1940; Suomalainen, 1940). In the aphids and phylloxerans, which arise by diploid parthenogenesis, the male has fewer chromosomes than the female, so that male production involves chromosome elimination, which yields a situation very similar to that represented by the $2x : 2A :: 1X : 2A$ relationship (Morgan, 1912, 1915). There is no great difficulty surrounding sex-determination in diploid parthenogenesis when diploid offspring are of the same sex as their diploid mother, but even though a great advance has been made with the discovery of Whiting in *Habrobracon*, haploid parthenogenesis still offers innumerable opportunities for research of great interest and importance (cf. Schrader and Hughes Schrader, 1931, Darlington, 1937).

IV. SEX-DETERMINATION BY EXTRA-ZYGOTIC (ENVIRONMENTAL) FACTORS OR BY A COMBINATION OF EXTRA- AND INTRA-ZYGOTIC FACTORS

In the rotifers there are two kinds of females, amictic and mictic. In the amictic female reproduction is always by parthenogenesis and she produces only females which may be amictic like their mother or mictic. These mictic daughters may reproduce parthenogenetically, in which case they yield only males. But if they are impregnated they produce resting eggs, fertilised eggs. From these females appear and they are invariably amictic. In the next or one of the following generations mictic females appear again.

But the work of Luntz (1926) would appear to show that in Rotifera the alternation of generations is a response to extra-zygotic factors. If the environmental conditions are kept constant the rotifers reproduce parthenogenetically to yield only females. Variations in the pH, changes in nutrition or in the concentration of the culture medium, regularly influence the females in such a way that their eggs in the following generation develop into mictic females.

In Cladocera the situation is even more complicated and confused. It would appear from the results obtained by Banta and his co-workers (1929a, 1929b, 1929c, 1930), by Berg (1934) and by Tauson (1930), that here also environmental factors are the more important in sex-determination. Fertilised eggs yield females, unfertilised eggs yield both males and females, but whether male-determination and

female-determination are the results of the impress of environmental factors on a genetic mechanism or upon physiological processes is not known.

Banta and Brown (1929a) found that crowding of parthogenetic females favoured male-production, which could be counteracted by such procedures as agitation of the medium or by aeration which might involve redistribution of food, increase of available food, or increased activity on the part of the Cladocera exposed to them. They observed a retardation of the development of the mothers proportional to the degree of crowding. The same retardation was produced by the addition of certain chemicals to the medium, probably by lowering the metabolic rate. When by chemical means an increased rate of development was evoked, an approximate inverse proportionality between the rate of development and the percentage of males was found (Banta and Brown, 1930). These results are in contrast with those obtained by Tauson (1930), but she used females which already had eggs or embryos in the brood pouch and since the sex of the eggs in *Moina macrocopa* with which Banta and Brown were working is determined four hours before the egg passes into the brood pouch, it may be assumed that the same holds true for *Daphnia pulex*, with which Tauson worked, and that the offspring of the treated females came from eggs the sex of which was already decided. The further development of the eggs may have been prevented by the chemicals employed.

Tauson also studied the influence of temperature, nutrition and acidity on sex-determination in *Daphnia pulex*. At 15°–25° C. parthenogenesis is the rule, below 15° C. sexual reproduction. When the temperature is reduced below 15° C. males first appear and later gamogenetic females. Insufficient nourishment induces the appearance of males followed by ephippial females. At pH of 6.7–6.3 males appear in considerable numbers; with increasing pH females appear in increasing proportions until at length only females are produced. It would seem to be the case that both in the Rotifera and the Cladocera a depressed metabolic rate is responsible for the change-over from parthenogenesis to sexual production.

The marine worm *Dinophilus apatris* provides an excellent example of ovum dimегaly (Korschelt, 1882), a condition which is also present in mites (Reuter, 1907) and spiders (Montgomery, 1907), as well as in the parthenogenetic eggs of several genera of rotifers and phylloxerans. Riddle (1917) maintains that in the pigeon also there are two kinds of eggs, a smaller with higher water content and lower energy content as measured by the amounts of fat and phosphorus, and a larger with lower water content and higher energy content. The smaller egg is, according to him, male-producing, the larger female-producing. In the rotifers, if the egg is fertilised it becomes a large egg and a female, if not it remains a small one and yields a male by haploid parthenogenesis. In the phylloxerans both large and small eggs are parthenogenetic, but whilst the large ones retain the full diploid number of chromosomes during maturation, the small ones extrude an X-chromosome to assume the male constitution. The sex-chromosome itself is not the sole determiner of sex here but acts only as a differential in a complex reaction system, for the eggs are predestined as male-producing and female-producing irrespective of their chromosome content, whereas sex itself is not determined until during the maturation of the egg, when one or other type of chromosome constitution becomes established. Since the eggs are predestined as male and female before the elimination of the X element to yield the characteristic male

chromosome constitution, Morgan (1912) concluded that sex itself is determined already in these eggs before they are laid (progamic sex-determination). In *Dinophilus* the females produce large "female" eggs and small "male" eggs. Both kinds are fertilised and reduction and fertilisation do not appear to have any influence on sex-determination. Hartmann (1933) has shown that the ratio of male to female eggs can be altered considerably by environmental factors, that unimpregnated females from isolated eggs produce both kinds of eggs, and that in a certain race unfertilised eggs can develop parthogenetically to yield both types of eggs; facts which prove conclusively the existence of sex-determination by external agencies.

For twenty years and more Baltzer (1935, 1937a, 1937b) has been investigating the astonishing facts concerning sex-determination in the marine worm, *Bonellia*, which is remarkable for its pronounced sex-dimorphism. The female has a green body of the size and shape of a walnut, with an enormously long proboscis sometimes as much as a yard in length. The male is minute, 1-3 mm. in length, and lives as a commensal partner in the female's intestine and uterus. The sex-ratio among these worms is 10-20 males to each female. The young green-spotted larvae are free-swimming for the first ten days or so and thereafter sink to the bottom of the waters. Usually it would seem that their sexual destiny is decided by accident. If a larva settles on or near a female, it develops into a male; if, on the other hand, it avoids a female, it becomes a female. This being so, it seems quite clear that there is no intra-zygotic sex-determining mechanism in the creature, or that, if there is, it is of little account, and that there must be something provided by the adult female which irresistibly provokes male-type differentiation in the young larva. But sex-determination in *Bonellia* is not quite so straightforward as it appears at first sight. Baltzer and others have studied the problem experimentally, and have succeeded in showing that what seemed simple is really most complicated. If, for example, representative samples of these free-swimming larvae are taken and some placed in ordinary sea-water and some in identical sea-water to which pieces of the proboscis of the adult female have been added, it is found that of the first lot about 5 per cent. become males or intersexual forms, the rest developing as females, whilst of the second batch some 70 per cent. develop into males and the rest into females. The same result is obtained if extracts of proboscis are used instead of the proboscis itself; so that there arose the notion that the proboscis elaborated some kind of hormone—a male-differentiating hormone. Next it was found that certain quantities of acid or of copper added to the water increased the percentage of males, possibly through altering the oxidation rate in the larvae.

The larva, when it becomes attached to the female proboscis, does so by means of a sticky secretion provided by the ventral surface of the anterior half of its body. The male-differentiating influences apparently pass across this mucous bridge. By leaving the larva so attached for shorter or longer periods of time, it is possible to produce intersexual forms, male-type in the anterior (attached) half and female-type posteriorly. When intersexes are produced by the addition of extracts of proboscis to the sea-water, again it is the anterior half that is male-like, the posterior female-like. Perhaps the male-differentiating substances enter the larval body by way of the glands on the anterior half of the ventral surface, and thence diffuse throughout the body or maybe the anterior half is more

permeable than the posterior. On the other hand, it may be that there are different thresholds of response at different levels of the body.

But the real difficulty is created by the fact that both under natural and experimental conditions all larvae exposed to the same stimuli do not react in the same way and to the same degree. Baltzer has been driven to the conclusion that genetic differences between the individuals concerned are responsible for this disagreement, and that in *Bonellia* sex is not determined by extra-zygotic factors alone, but by the interaction of certain varying environmental factors with different genetic predispositions; there are external agents that encourage male-differentiation, others that favour female-differentiation; there are larvae so constitutionally equipped as to develop into males without any encouragement, whilst others, for this achievement, demand strong environmental reinforcement.

V. SEX-DETERMINATION IN THE AMPHIBIA

The study of *Drosophila* and *Lymantria* has shown that gonochorists (species in which ovaries are found in one individual, testes in another) are amphisequential, and that they follow a unisexual course of development through the suppression of one sex potentiality, expression and suppression being determined by the balance or imbalance between male and female-determining genes. *Drosophila* has proved to be a most excellent material for the genetical study of the problem of sex-determination, whilst *Lymantria* has made notable contributions to our knowledge of the ways in which the genes produce their effects. The amphibian, especially the frog, has much to add to what *Lymantria* has already given. Following upon the observations of Crew (1920, 1921) and of Witschi (1922, 1928a, 1928b, 1929) that the fairly common adult hermaphrodite frog was, in fact, a female undergoing sex-reversal, Witschi (1934) examined frog populations from different localities and found that there were geographical races in which sex-differentiation was complete in the early larval stages, there being among these males and females and no intermediates, whilst in other races juvenile hermaphroditism (first described by Pflüger in 1882) was very prevalent. The frog is really a protogynous hermaphrodite, and the different geographical races differ among themselves in respect of the duration of the female phase. Thus, in the Freiburg race all animals at the time of metamorphosis are in the female phase, but during late summer 50 per cent. of them undergo sex-reversal to become males. In some individuals this sex-transformation does not occur until the individual is fully adult and has actually functioned as a female. Alpine and Baltic races, on the other hand, have an exceedingly short female phase so that the males are already male in the larval stages.

The frog thus forms a link between the gonochorist and the true hermaphrodite (between the dioecious and the monoecious species). Genetical studies have shown, though not entirely conclusively, that the male is XY, the female XX. No sex-chromosomes can be detected, cytologically, in amphibia. Witschi (1914) found a gene *f* in the Y-chromosome of the frog. The *f* gene is the allele of *F* in the X and plays a distinct rôle as a quantitatively reduced female-determining agent, having a lower value in the races in which sex-differentiation occurs relatively early in larval life. Witschi gives the formulae for male and female as

MMFf and MMFF respectively, the M genes being situated in the autosomes, F in the X-chromosome and f in the Y.

Witschi is of the opinion that this MMFf : MMFF mechanism has been derived from the hermaphroditic MMFF, in which MM = FF, as a result of quantitative changes in the female genes, these diminishing in the Y-chromosome and increasing in the X to give the fY and FX relationship. The relatively high and low values of the Y-borne female-determining genes in the different geographical races are regarded as evidence of this transition from the hermaphroditic to the gonochoristic type in which $f = 0$ and in which the Y-chromosome is either inert or else has disappeared.

He offers an attractive suggestion which attempts to relate genic action to developmental physiology, to link up sex-determination and sex-differentiation. The mode of differentiation of the germ cells is imposed upon them from without ; no matter what their genetical constitution may be, they become oocytes if under the influence of the gonadic cortex, into spermatocytes if under the influence of its medulla. Cortex and medulla are the inductors of sexual differentiation, and both are present in the embryonic gonad. The female-determining and male-determining genes respectively cause the appearance of the cortical and medullary inductors (cortixin and medullarin). In genetic males (MMFf) the corticular development is relatively suppressed ; in genetic females (MMFF) it is " stronger " than the medulla and dominates the processes of sex-differentiation. This attractive hypothesis, which is in harmony with that of Goldschmidt, is well founded in the results derived from work on parabiosis and embryonic grafting. If a male and female toad are grafted together during the early stages of embryonic development and thereafter allowed to grow until sexual differentiation is well advanced, examination will show that each of these parabiotic twins has pursued a development unaffected by its partner. But if frogs are used instead of toads the ovary nearest the male is suppressed or even partially transformed into a testis-like body. In the case of salamanders and newts the relative sizes of the partners affect the results. If the male is much smaller than his female partner his gonads become ovaries ; if the male and the female are equal in size or if the male is the larger, then the ovarian elements in the female are more or less completely suppressed and testicular development in the male is somewhat retarded. The differences in effect are referable to differences in the diffusibility of the substances concerned.

In 1931 Humphrey reported the successful reversal of sex in the salamander *Ambystoma*. This led to the hope that evidence as to the mechanism of sex determination in this genus might be obtained by mating such transformed animals with normal ones. It soon became evident that whilst the transformation of female into male could be accomplished with relative ease in *Ambystoma tigrinum*, animals of this species could rarely be bred in the laboratory. However, one such successful mating was secured and amongst the offspring of this there was a considerable number of males, which seemed to mean that the female was not homogametic. This interpretation was made difficult by the uncertainty as to whether or not the normal spawning female might have retained spermatozoa from previous matings with normal males and secondly that germ cells of the male donor, included in the orthotopic graft which had induced reversal, might have become incorporated in the ovary of the host.

Because of these difficulties the Mexican axolotl (*Siredon mexicanum*) was used in order to secure sexually transformed animals. The transformed females were found to be sterile, although perfectly normal spermatozoa were actually present in their gonads in great numbers, and also in spite of the fact that the endocrine function of these testes was also normal. The sterility resulted solely from the fact that previous to reversal the continuity between the rete-cord material in the mesovarium and that in the ovary had become interrupted.

But in 1941 a transformed female in which this discontinuity was not present was obtained and this animal proved to be completely fecund and fertile in mating. Mating of this animal with two females produced both males and females. When the percentage of males in the offspring of the transformed animal was compared with that in the offspring of closely related males it was found to be decidedly lower. Only 26.1 per cent. of the young were found to be males as compared with 47.1 per cent. in the case of related males.

A few other reversed animals were found capable of reproduction. These included six females (one *Ambystoma tigrinum*, five *Siredon mexicanum*) converted into males through reversal of their remaining ovary under the influence of a testis graft replacing their right gonad, and one genetic male (*Siredon mexicanum*) whose only gonad was a testis resulting from the reversal of a graft ovary supplied by a *tigrinum*-*Mexicanum* hybrid. Offspring were obtained from two of the above animals by use of semen from the *vas deferens* connected with the reversed gonad to inseminate eggs removed from the oviducts of spawning females. The other five animals participated in twelve successful matings with as many normal females.

The F_1 offspring of each of these seven animals always included males. The lowest percentage of males was 17.3, the highest 35.3, the average was around 25 per cent.

Three generations of the progeny of the transformed axolotl (*S. mexicanum*) were studied. Of the F_1 offspring all males and about two-thirds of the females produced offspring consisting of males and females in approximately equal numbers. The remaining third of the females of the F_1 produced only female offspring. Of these, twelve, whose fathers were normal males, all produced both male and female offspring, but of the fourteen whose fathers were reversed females five produced only females. The results obtained did not accord with the postulate that the female is heterogametic. A reversed female XY mated with a normal female XY should produce offspring in the proportion of 1XX, 2XY, 1YY or 25 per cent. males if the YY genotype develops into a female. Such YY females constitute one-third of the total number of that sex and should produce only female offspring since they lack the X-chromosome which a normal female would transmit to her sons (see Humphrey, 1942, 1945).

VI. SEX-DETERMINATION IN FISHES

The fish has been used extensively in investigations relating to sex-determination. Kosswig (1935, 1936, 1937) and Breider (1935a, 1935b, 1936) have worked with *Xiphophorus*, certain species of *Platyocilus* and of *Limia*; Winge (1932, 1934) with *Lebistes* and Aida (1936) with *Aplocheilus*. Genetical evidence obtained by Gordon (1937) and by Bellamy (1936) has shown that in *Platyocilus maculatus*

the female has the XX constitution. It is known that juvenile hermaphroditism is the rule in *Limia*, and that sex-reversal from female to male is not uncommon in *Xiphophorus*.

The results obtained by Kosswig and by Breider are of great value and interest, but it has to be confessed that the hypotheses presented to explain them are most complicated and confusing. They appear to dislike the *Drosophila* and *Lymantria* models of the sex-determining mechanism and construct a new one for themselves. This, to accommodate all the observations they have made, sometimes demands that there shall be no intra-zygotic sex-determining mechanism at all, that in some fish the male shall be XY, in others XX, that in some cases the X-chromosome shall be empty or that the female-determining genes shall be in the Y, and that there shall be multiple autosomal male-determiners. There is every reason why new hypotheses should be constructed when once it has been shown that already existing and tested ones are not satisfactory, but, as Goldschmidt (1937) has suggested, all the results obtained can quite readily be accommodated by the ordinary scheme according to which $FFMm = \text{♀}$ and $FFMM = \text{♂}$. M being in the X-chromosome, the Y being inert, and F in the autosomes, the usual balance being $FF > M < MM$. The *Limia* and *Xiphophorus* results can be explained by an appeal to Witschi's hypothesis concerning sex-determination in the frog.

Winge found the male of *Lebistes* to be XY according to genetic evidence. The Y carries several colour genes, some of which can become transferred to the X by crossing-over. Among the many important observations made was that XX males, recognised by the genetic evidence they presented, occasionally made their appearance. Matings to ordinary XX females, produced female offspring exclusively, but, in later generations (the mating of different XX males to their female relatives) yielded different sex-ratios among the progeny. One and the same mating would give both males and females in summer, females only in winter. Winge also obtained males that, according to the genetic evidence, were YY, and these, mated to ordinary females, produced males only.

Winge presents the view that in *Lebistes* there is a potent female-determining gene in the X and a strong male-determining gene in the Y, and that the autosomes carry many male-determining and female-determining genes of different values. Ordinarily these autosomal genes are balanced ($F = M$) and so cancel each other out, so that sex-determination is decided by the balance between the sex-chromosome-borne genes. But he is of the opinion that, by selection, it is possible to collect in an individual a sufficient number of autosomal male-determining genes, or alternatively of female-determining genes, of such potency that the autosomal male- or female-determining genes are more powerful than are the sex-chromosome male- or female-determining genes. The real arbiter of sex is now the autosome—it is now the real sex-chromosome, and the original sex-chromosome, robbed of its importance, sinks to the level of an autosome. The XX male, for example, is an individual in which the sex-chromosomes no longer dominate the processes of sex-determination; it is one in which autosomal male-determining genes take charge.

This suggestion is most attractive; it seems to offer an explanation of the puzzling fact that, whereas the male is heterogametic (XY) in some forms, it is homogametic (XX) in others. There is nothing very extravagant or inherently improbable in Winge's argument. Between Winge and Goldschmidt there is

conflict, for Goldschmidt (1937) holds that all Winge's results can be accommodated by the Lymantria model of the sex-determining mechanism. Winge (1937) on the other hand, objecting to Goldschmidt's suggestion that the female element of the sex-determining mechanism is carried in the cytoplasm, maintains that all Goldschmidt's results can be explained if it be assumed that in Lymantria, as elsewhere, there are multiple sex genes in the autosomes, some being male-determining and others female-determining.

Aida, working with *Aplocheilus*, has obtained results that are very similar indeed to those of Winge. The male is XY and both X and Y carry colour genes. XX males appear and in different matings yield different sex-ratios. Aida also got XY females, which mated to XY males, gave the expected 3 males : 1 female ratio. The YY males were viable and when mated to XX females produced male offspring only. Aida proffers a theoretical explanation of his results which is essentially that already elaborated by Goldschmidt and Witschi in connection with *Lymantria* and the frog.

It is clear that heterogamety and sex are two quite distinct phenomena, even though they may be produced by one and the same mechanism. It now seems to be entirely reasonable to hold the view that the phenotypic sex of the individual is normally in harmony with the sex-chromosome constitution for the reason that the really decisive elements of the sex-determining mechanism are resident in the X. The X-borne genes, which in different cases may be male-determining or female-determining, are present in the heterogametic individual in the simplex condition, in the homogametic individual in the duplex state. They constitute the weight in the one pan of the scales. In the other are the genes of the opposite kind, in the Y or in the autosomes. The two kinds, M and F, are present in a given individual in quantitatively different proportions so that either $M > F$ or $F > M$. In the autosome also are other genes which are concerned in sex-determination, being male-determining or female-determining. Their weight is added to one or other of the two pans of the scales, but, as a rule, their addition does not materially affect the result, but merely makes this more decisive. However, they may mutate and then disturb the balance to produce intersexuality. During the evolution of the chromosome there has been a progressive accumulation of genes of the same kind in respect of male-determination and female-determination in the X-chromosome and the others respectively.

Instead of these genes differing among themselves in number, they differ, according to the views of some, in respect of valency and potency. There are "strong" and "weak" male-determining and female-determining genes. If this is the case, it becomes possible for an individual to possess a sex-chromosome constitution that is not in harmony with its ultimate sexual characterisation. Such an individual is usually classified as an instance of sex-reversal, which actually it is not, since it was never a female that became a male or a male that became a female. It began its career with a certain chromosome constitution, XY or XX, which is characteristic of the male or female of the species, it had the usual number of male- and female-determining genes appropriate to an individual with such a chromosome constitution, but, owing to the unusual relative valencies of these genes, it pursued its development under the direction of that sex-differentiating stimulus which normally, in individuals of this chromosome constitution, is dominated by the alternative.

If the relation of these two genes or gene complexes, M and F, can be disturbed by the addition to one side of the equation of other subsidiary or modifying genes, then it is not surprising that the rate of production by them of those substances which take charge of differentiation can be influenced by environmental forces, or that the action of these substances should be disturbed. It should be possible, theoretically, in forms in which the sex-determining mechanism is not too thoroughly insulated, to influence this production, and in this way to affect sexual differentiation.

VII. SEX-DETERMINATION IN BIRDS

The genetic evidence points directly to the existence of the typical XY : XX (or XO : XX) sex-determining mechanism, the male being XX, the female XY or XO. Typical sex-linked plumage characters have been found in a variety of forms, e.g. silver, barring and non-barring in the fowl ; the narragansett pattern in the turkey ; cinnamon colour in the canary ; cinnamon, opaline, slate and lutino in the budgerigar ; and wherever these are found they invariably indicate that in all forms this rule holds. The cytological evidence is not so convincing owing to the difficulties presented by the very large number of chromosomes, many of these being minute, but, as far as it goes, it is in harmony with the genetical. White (1932) and Crew (1933) agree that the male of *Gallus domesticus* has the XX constitution, and more recently Miller (1938), taking advantage of the fact that following ovariectomy in a few-days-old chick, a testis-like organ, in which active spermatogenesis is later encountered, develops on the right side of the body at the site of the vestigial right gonad, was able to compare the spermatogonia and spermatocytes of such sex-reversed females and of normal males. He identified the X-chromosome and since sex-reversal of this kind does not affect the chromosome constitution of the gametes that are elaborated, he was able to show that the female has but one X-chromosome, the male two (Suzuki, 1939).

The same is true of the pigeon and the ring-dove. Painter and Cole (1943) have shown that when male pigeons are crossed with female ring-doves nearly all the offspring which hatch are males. This disturbance of the sex-ratio is caused by a differential mortality of the female embryos. The diploid chromosome number of the pigeon is about 68 ± 4 and the female is heterogametic. The chromosome complexes of the ring-dove are indistinguishable from those of the pigeon. In about 49 per cent. of the fertile eggs produced by a pigeon - ring-dove mating the embryos die during the first week in the incubator. A characteristic feature of the embryos which are to die is the presence of a conspicuous blood ring. Such embryos with blood rings examined cytologically show a single X-chromosome and are therefore to be regarded as genetic females.

The fowl has made another contribution towards our understanding of the sex-determining mechanism, and this has emerged from a study of lateral asymmetry and gynandromorphism. Crew and Munro (1937, 1939) examined cases of mosaicism to find that all these birds showed lateral differences in respect of size, those in the fowl falling into two classes : (1) that in which the difference between the two sides was around 2 per cent., and (2) that in which this difference was around 10-15 per cent. The genetic evidence supplied by these cases showed clearly that the lateral asymmetry in the birds in the first class was due to the

elimination of an autosome on the smaller side, and that in the birds of class two non-disjunction of an autosome had occurred, resulting in bilateral heteroploidy with a gain of this autosome (AAA) on one side (the larger) and a loss on the other (A). The fowls in class one were either males or females showing no kind of sex abnormality, but several of the second class were gynandromorphs, whilst others were males. Apparently the birds with the usual male equipment were genetic males (XX) while the gynandromorphs, with testis on the right and ovary on the left, were genetic females (XY). It was clear that this gynandromorphism was not the result of the elimination of an X-chromosome; undoubtedly an autosome was concerned for it could be identified by means of certain flesh-colour genes resident in it. Warren (1945) describes a further case of the class two type.

These cases seemed to show that if in a genetic female non-disjunction of a particular autosome (the one associated with the autosomal genes for white and yellow flesh) occurred to yield a gain on the right and a loss on the left the disturbance in the genic balance in the tissues on the right was such as to provide the necessary stimulus for the development of the right gonad, which in the normal female remains quite rudimentary, and that under these circumstances the right gonad developed as a testis. It further seemed that if in a genetic male there is a loss of this autosome on the left side then the disturbance is such as to lead to the development of an ovo-testis on this side.

But in the light of the work of Domm (1924, 1927, 1929), Benoit (1923), Greenwood (1925) and others, it is not easy to ascribe these abnormalities in respect of gonadic structure to such maldistributions of the chromosome. Domm, for example, showed that if a female chick is ovariectomised shortly after hatching, the removal of the ovary allows the rudimentary right gonad to resume its development. It becomes a testis-like organ. It is reasonable to hold the view therefore that so long as the functional ovary is present the rudimentary right gonad cannot continue to develop. But how is it that when this inhibiting factor is removed the right gonad develops into a testis? It has the same chromosome constitution, the same genetic constitution as has the left gonad, yet one develops as an ovary and the other, when it is permitted to do so, develops into a testis. If we adopt Witschi's scheme for the linking up of sex-determination and sex-differentiation by assuming that the genetic forces M and F act on the medullary embryonic gonad then it can be assumed that the right gonad of the female does not react to F because it has no cortical elements in the composition. How then is its renewed development following upon the removal of the dominant functional ovary to be explained? Is it the case that, as Crew (1923) suggests, the relationship of M and F undergoes a switchover shortly after the gonads are differentiated so that in both sexes after hatching $M > F$? If this were so, then any embryonic gonadic tissue still capable of responding would differentiate under the direction of M.

This question brings us into one of the most exciting fields of experimental inquiry. The work of Wolff and Ginglinger (1935), Dantchakoff (1936) and of Willier, Gallagher and Koch (1937), in which hormones were injected into the incubating egg, has shown that male hormone can cause the disappearance of the cortical elements of the gonads wherever such exist and that female hormone can cause the disappearance of the medullary elements and that at the same time each provokes a fuller development of the homotypic tissues.

By these means the embryonic gonads have been explored. It has been found that in the male, the right gonad includes fewer cortical elements than does the left whereas in the female whilst the left gonad consists almost entirely of cortical tissue, the right one has practically no cortical elements at all. It is not surprising therefore, that the two kinds of embryonic gonad, embryonic testis and embryonic ovary, react differently to the M : F genetic mechanism, and that the two gonads in one and the same bird also react differently thereto. The kind and the degree of their reaction is determined by their own architecture.

This argument is based largely upon the conjecture that the gene products M and F are chemically and/or physiologically similar to the adult hormones used in these experiments in which it has been shown that female hormone injected into an egg with a male embryo converts the left embryonic testis into an ovo-testis or else into an ovary, according to the dosage, whilst the right embryonic testis is left unaffected. If, on the other hand, the embryonic gonads of a genetic female are exposed to the action of the male hormone, the right gonad develops into a testis with moderate dosage, whereas to produce this effect in the left requires a much higher dosage and even then the suppression of the cortical elements is never complete.

Differences in respect of anatomical relationships may perhaps be the cause of these differences in the composition of the two gonads of one and the same bird. This, of course, is a problem in developmental physiology, not in genetics. But certainly laterality is a factor of considerable importance in this connection, the two gonads of one and the same individual are not alike ; not alike in their morphology ; not alike, therefore, in their capacity to respond to male and female hormones and presumably also to the gene products M and F.

It may well be that these gene products are in charge of sex-differentiation up to nearly the end of the incubation period, that they cease to operate when once the hormones of the differentiated gonad have taken over from them and that the development of a testis on the right side of the ovariectomised female is of the nature of simple growth, the gonad becomes larger and more differentiated in the only way possible to it since it is a medullary derivative. Maybe it is for this reason too that the result of the maldistribution of chromosomes is to throw the M : F mechanism out of gear and to permit any gonadic tissue that can develop to do so, medullary derivatives on the right in the female, cortical derivatives on the left in the genetic male.

This hormone injection work has shown quite clearly that sex-reversal in either direction can be induced in the chick. Specific hormones act upon definite embryonic rudiments which react in accordance with their own special abilities. Every embryo, genetic male and female alike, possesses all the primordia necessary for the realisation of either type of sexual equipment. These primordia are of two kinds, those which pursue one or other mode of differentiation, e.g. the external genitalia which develop either according to the male plan or else to the female plan, and those which are present in pairs, one of which achieves full development whilst the other remains embryonic in its dimensions, e.g. the Müllerian and Wolffian ducts. It is reasonable to expect that in any given individual it will be much easier for the rudimentary apparatus proper to the other sex to be provoked to further development than for the differentiated external genitalia to undergo any considerable remodelling. Thus the possibility of sex-

reversal is determined largely by the difference between the differentiated external genitalia of the two sexes. In the amphibian, fish and fowl, this is not great and therefore complete sex-reversal is possible. The gonads are composed of cortical and medullary elements ; one of these can be suppressed and the other provoked to fuller development ; the rudimentary Müllerian or Wolffian ducts can assume full proportions and the cloacal differences between male and female are commonly merely differences of degree and not of kind. The situation is very different in the mammals, in which the external genitalia of the two sexes differ so very markedly.

VIII. SEX-DETERMINATION IN MAMMALS

The cytological evidence so far accumulated points directly to the conclusion that in mammals without any exception the male is heterogametic, the female homogametic. *Homo sapiens*, Painter (1923), Evans and Swezy (1929) ; Koller (1937) ; *Sus scrofa domesticus*, Crew and Koller (1939) ; *Rattus norvegicus*, Koller and Darlington (1934) ; *Mus musculus*, Painter (1927) ; *Lepus formosus*, Tateishi (1936) ; *Apodemus speciosus*, Oguma (1934) ; *Evotomys bedfordiae*, Oguma (1934) ; *Evotomys glareolus*, Matthey and Renaud (1935) ; *Didelphys virginiana*, Painter (1922) ; *Pecari tajacu*, Krallinger (1936) ; *Microtus montebelli*, Oguma (1937) ; *Cricetus auratus*, Koller (1938) ; *Cricetulus griseus*, Pontecorvo (1943b) ; *Dasyurus maculatus*, *Sarcophilus ursinus*, *Phascolarctus cinereus*, *Pseudochirus peregrinus*, *Trichosurus vulpecula*, Koller (1936 a) ; *Sciurus carolinensis leucotus*, Koller (1936 b) ; *Talpa europea* and *Putorius furo*, Koller (1936 c).

Genetic evidence concerning the sex-determining mechanism is scanty. In man alone among the mammals, several sex-linked genes are known and their mode of transmission makes it quite certain that the male is heterogametic. Indeed, a map of the X-chromosome of man is beginning to take shape (Haldane, 1936b ; White, 1940). Differences between the species in respect to the relative length of the differential segments of the sex-chromosomes do not seem to be sufficient to account for the fact that only one sex-linked gene is known in mammals, other than man ; i.e. in the cat (Pontecorvo, 1943b). It would seem that the evolution of the sex-chromosomes in mammals has taken the form of a progressive specialisation, the differential segment of the X coming to carry almost exclusively female-determining genes and the pairing segments of the X and Y male-determining genes ; if this is the case then it is the explanation of the rarity of sex-linked characters in the mammal (Pontecorvo, 1943b).

Abnormality of the reproductive systems taking the form of an apparent or actual mixture of male and female structures, has frequently been reported in mammals, more often in man and his domesticated animals than in wild species for reason, presumably, that the former types are under closer observation and are more commonly dissected. There is no doubt that the tendency to throw intersexual offspring is inherited, but to disentangle the genetic causes of this condition from secondary effects is at present beyond our powers.

Crew (1923a, 1924), who examined a number of cases in the goat and pig, suggested that the individuals were genetic males in which the gonadic hormones that stimulate the male primordia to develop and inhibit the growth of the female primordia are produced too late in development or are produced in too small

quantities at the critical times. Baker (1925, 1928) accepted this suggestion as a possible explanation of the abnormalities he encountered in the sex-intergrade pig of the New Hebrides, but presents the view that in the ordinary cases of intersexuality it is more probable that in its earlier stages of development the individual is equipped with both ovarian and testicular tissues and that the differentiation of the various organs takes place under the antagonistic influence of the male and the female sex-hormones. This hypothesis is more in harmony with the theories of Goldschmidt and of Witschi and with the views of Krediet (1929) who has made a special study of this subject and whose paper should certainly be consulted by such as seek a really critical review and a comprehensive bibliography. Krediet presents the view that the distinction between the pseudo-hermaphrodite (abnormal only in respect of the derivatives of the Müllerian and Wolffian ducts and of the external genitalia) and the true hermaphrodite (with a mixture of ovarian and testicular tissues) is unreal, for the reason that both are but stages in one and the same process of sex-transformation. He uses Goldschmidt's theory of intersexuality in explanation. Sex-reversal in the mammal can, he maintains, proceed in the direction $\text{♀} \rightarrow \text{♂}$ in the indifferent stage of the gonad, in the stage when the medullary cords become differentiated, in the stage when the cortical cords are differentiated and also after birth. In the direction $\text{♂} \rightarrow \text{♀}$ it can occur during the first two of these stages. Krediet prefers to think that the intersexual gonad elaborates an intersexual hormone and not both male and female hormones.

In the case of the goat, Asdell (1944) has produced evidence of very considerable weight which points to the conclusion that the intersexual animal is a genetic female, that intersexuality of this kind is definitely genetic in nature and that its gene is closely linked with that which yields hornlessness. He records that in the Beltsville herd of goats the Saanen breed produced 11.1 per cent. of intersexes and the Toggenburgs 6 per cent., whilst in the British Saanen population the percentage of intersexes was no less than 14.3. The secondary sex-ratio amongst these goat populations was as follows: For Saanens 49.3 per cent. males, 39.6 per cent. females and 11 per cent. intersexes; for the Toggenburgs it was 46.4 per cent. males, 47.6 per cent. females and 6 per cent. intersexes; amongst the British Saanens there were 193 males, 105 females and 52 intersexes. Asdell points out that the sex-ratio in all these cases is much nearer normal if the intersexes are recorded as modified females and argues that this suggestion is in line with the fact that in vertebrates modification of sex is almost always in the direction of female to male. Asdell has encountered some 200 intersexual goats and so far has failed to encounter a single case in which the animal possessed horns. He points out that if intersexuality of this kind is a simple recessive, hornlessness being a simple dominant, and if the two are closely linked, then selection for hornlessness, which has been exercised by pedigree goat breeders for a considerable time, might quite easily result in an increase in the frequency in a population of the gene for intersexuality. This could explain the observed fact that intersexuality in the goat is increasing to become a problem of serious magnitude. It is indeed a matter of some importance to the industry to decide whether the intersex is a genetic male or female for the reason that the loss of a male through this cause is not a matter of any great moment, whereas the loss of females can lead to economic disaster.

IX. THE SEX-RATIO

If in any species with a genetic sex-determining mechanism, one sex being heterogametic (XY or XO) and the other homogametic (XX), if the two forms of gametes (X and Y, or X and no X) are elaborated by the heterogametic individuals in equal numbers, if these two forms are equally capable of fertilising or of being fertilised, and if fertilisation takes place at random, that is to say, if the chances that either of the two forms of gametes elaborated by the heterogametic sex will unite with any particular gamete provided by the homogametic sex are equal, then among the zygotes resulting from fertilisation there should be equal numbers of homo- and hetero-gametic individuals; the primary sex-ratio should be equality. Any inequality of this primary sex-ratio would be referable either to an unequal production of the two kinds of gametes elaborated by the heterogametic sex or else to an unequal participation in fertilisation on the part of these two kinds of gametes.

If the secondary sex-ratio, that which obtains among the newly-born, differs from the primary sex-ratio, then a sexually selective prenatal mortality must have been operating between conception and birth.

In those species in which sex-determination is established by extra-zygotic factors, the primary sex-ratio may be expected to be anything from 0 males : 100 females, to 100 males : 0 females, according to the kind of extra-zygotic factors concerned and to the intensity of its action.

But since the secondary sex-ratio among the species with the homo-heterogametic genetic sex-determining mechanism is never equality though usually not far removed therefrom, certain questions present themselves. If the divergence from equality is statistically significant, are fewer Y-chromosome-bearing spermatozoa produced, is the Y-bearing sperm handicapped in fertilisation, or is the XY zygote less viable than the XX and, if so, what is the reason for this? In certain *Drosophila* stocks it can be conclusively shown that the Y-bearing sperm is either not produced in numbers equal to those of the X-bearing or else if they are they are not capable of fertilising the eggs. Sturtevant (1925) and Gershenson (1928) discovered a gene which in its action leaves the female unaffected, but the sex-ratios of progenies sired by a male with this gene vary from 85 to 100 per cent. females. It was demonstrated that these disturbances of the sex-ratio were not due to any differential mortality. Later, Sturtevant and Dobzhansky (1936) found this "sex-ratio" gene widespread in different geographical races of *Drosophila pseudo-obscura*. It is resident in the right arm of the X-chromosome and is associated with a small inversion. In its action it induces equational division of the X-chromosome at each meiotic division whilst the Y-chromosome degenerates. The result is that only X-bearing spermatozoa remain available for fertilisation.

In forms which have not been subjected to the same intensive genetical exploration it is impossible of course to relate the non-appearance of one of the two expected types of gametes to the action of a particular gene of this kind. Nevertheless it is reasonable to suspect their presence. In *Aphis saliceti* Baehr (1908, 1909) found that the female always has 6 chromosomes, the male 5. In the egg there are 3 and in the sperm there should be 3 or 2. Fertilisation should yield females with 6 and males with 5. Actually, however, only females with 6 appear

from fertilised eggs. The reason for this is that in the first maturation division of spermatogenesis the normal separation of the chromosomes occurs so that one daughter cell receives 3 and the other 2, but the latter degenerates and disappears so that only the 3-chromosome bearing sperm remain to yield 100 per cent. females on fertilisation. Morgan (1908) disclosed the same phenomenon in the phylloxerans in which only the X-bearing sperm complete their development so that females only result from fertilisation.

Thus it is seen that the primary sex-ratio may become removed more or less from equality as the result of a differential production of the two kinds of gametes elaborated by the heterogametic sex owing to an early removal by degeneration of one kind during gametogenesis. Another cause of unequal production would seem to be a constant or preferential orientation of the sex-chromosomes on the spindle of the maturation of the ova of the heterogametic female. Vandel (1938), who has made a study of the sex-ratio in the terrestrial isopod *Trichoniscus (spiloniscus) provisorius* Racovitza, finds that the females are either amphogenics, giving both male and female offspring, or monogenics, giving offspring which are wholly or preponderantly of one sex. The monogenics are to be classified into perfect arrhenogenics, giving only male offspring; perfect thelygenics, giving only female offspring; allelogenics, behaving successively as arrhenogenics and thelygenics; imperfect arrhenogenics giving a preponderance of males; imperfect thelygenics giving a preponderance of females; mixed, giving a sex-ratio of equality but which are out of monogenic mothers and whose daughters are themselves monogenics. The males exercise no effect upon the monogeny of the female and this would appear to be a property which exhibits a matroclinous mode of inheritance. Vandel assumes that the female is heterogametic and regards the arrhenogenic and thelygenic habits as reflections of two different states of the cytoplasm causing different types of movement on the part of the sex-chromosomes. By elimination of other possibilities he arrives at the conclusion that in the monogenic female the orientation of the hetero-chromosomes on the maturation spindle is constant and can be shown to determine the formation of ova all of the same sexual type. However, more recent work of Howard (1940) on the related genus *Armadillidium* suggests other possible explanations. The sex-ratio within the brood (one a year of 50 to 150) is determined by the female parent. Some broods are entirely monogenic, others show different sex-ratios, whilst very few consist of approximately 50 per cent. males and 50 per cent. females. Monogenic broods are interpreted on Vandel's (1940) hypothesis that the female is heterogametic and that the segregation of the sex-chromosomes is controlled by the cytoplasm.

This conclusion is in complete harmony with the findings of Seiler (1920) in the case of the psychid *Talaeporia tubulosa*. He was able to show that the ratio of the eggs in which the X-chromosome passed into the polar body to those in which it remained in the egg was exactly the same as the subsequent sex-ratio. Moreover, by varying the temperature during the disjunction of the chromosomes he was able to influence this process and so to disturb the sex-ratio in either direction. Vandel found that, using imperfect arrhenogenics or imperfect thelygenics, on increasing the temperature the proportion of females among the offspring decreased and that of the males increased, presumably for the reason that the X-chromosome remained more frequently in the egg under these conditions.

The egg of the heterogametic female contains the XY pair of chromosomes before polar body formation. Into the first polar body goes either the X or else the Y. If it is but a matter of chance which way this XY pair lies on the spindle then equal numbers of X-bearing and Y-bearing eggs will result. But if in a particular line this pair should habitually be so oriented on the spindle that the X passes into the polar body more frequently than does the Y then in this line there will be a preponderance of females; if, on the other hand, the orientation is such that the Y more frequently passes into the polar body the females of this line will be perfect or imperfect arrhenogenics. The sex-ratio amongst the offspring of single-pair matings of the body louse varies greatly, there being often a preponderance in the numbers of one or other sex and sometimes the broods are completely monogenic. These variations in the sex-ratio are not due to differential mortality of the sexes. The reasons for the present remain unknown (Hindle and Pontecorvo, 1942).

Yet another cause of inequality of the primary sex-ratio was encountered in the discussion concerning sex-determination in fishes. Winge and Aida have presented conclusive evidence that in species in which as a rule the male is heterogametic (XY), homogametic males (XX or YY) and heterogametic females (XY) can be created by appropriate genetical procedures. Their progenies necessarily disturb the sex-ratio and the degree of such disturbance will be determined largely by the proportion of these exceptional individuals among the parental generation.

These exceptional individuals are not to be regarded as instances of sex-reversal. They are males or females in which the phaenotypical sexual characterisation is in conflict with the usual, the standard, genetic sexual constitution of the species. They are homogametic males and heterogametic females of a species in which male heterogamety and female homogamety are usual. They should be not regarded as being in any way abnormal; they are merely exceptional because they are relatively few in a given population. They illustrate most clearly the reasonableness of the definitions of maleness and femaleness given at the beginning of this chapter and show that the morphology and physiology of the gamete are not related to its genetic constitution.

Actual sex-reversal, of course, can be directly responsible for a disturbance of the primary sex-ratio in the same way as can these exceptional males and females. The oyster (Orton, 1909; Gould, 1919); the sword-tailed minnow, *Xiphophorus helleri* (Essenberg, 1923); the millions fish, *Girardinus poeciloides* (Huxley, 1920); the frog (Crew, 1921; Witschi, 1922); the newt, *Triton alpestris* (Champy, 1921); the pigeon (Riddle, 1923); the fowl (Crew, 1923b), for example, have all provided well-authenticated examples of complete sex-transformation. The main effect of such sex-reversal is that whereas the individual formerly elaborated ova, it now produces spermatozoa or *vice versa*. But since its sex-chromosome constitution remains unchanged, its homogametic (or heterogametic) habit is unaffected. The sex-ratio among the progeny of an individual which has undergone complete sex-reversal will reveal the sex-chromosome constitution of the male and female of the species and will demonstrate the kind of sex-determining mechanism that is present. If, for example, a functional female should become transformed into a functional male and is mated to a normal female, and if among the progeny of this mating there are none but females, it can be concluded that in this species the female is homogametic and that the sex-determining mechanism is of the XY:XX type.

($XX \times XX = 100$ per cent. XX .) If, on the other hand, among the progeny there are either twice or three times as many males as females it can be accepted that the female is the heterogametic sex. ($XY \times XY = XX : 2XY : YY$.) The actual ratio will be determined by the viability or otherwise of the YY type. The YY male of *Lebistes* and *Aplocheilus* is viable; the YY individual in *Drosophila* does not appear.

Abnormal sex-chromosome number in the zygote is attended by a disproportionate production of the different kinds of gametes. The XXY , non-disjunctional *Drosophila* female, for example (*see* page 748) judged by the proportionality of the classes among her offspring, must produce more X and XY -bearing eggs than XX or Y -bearing eggs. That is to say, the three sex chromosomes disjoin more frequently between the X s (homeosynapsis) than between the X and the Y (heterosynapsis). This disparity is reflected in the primary sex-ratio. (Muller, 1925; Bridges, 1925; Stern, 1929.)

Polyploidy, the condition in which each chromosome, instead of being present in duplicate, is represented three, four or more times, is rare in animals though common in plants. Muller has suggested that the rarity of polyploid animals may be correlated with a disarrangement of the sex-determining mechanism since the few animal polyploids that do exist are either parthenogenetic or hermaphrodite.

Though the two forms of gametes elaborated by the heterogametic sex are produced in equal numbers, there will be inequality in the primary sex-ratio if the two kinds are not equally represented in fertilisation. Selective or preferential fertilisation by one of the two kinds of gametes is common in plants where there is a differential growth rate of the pollen tubes (*cf.* Brieger, 1930), but in animals such selective syngamy, if it occurs at all, is very rare. A number of attempts have been made to determine whether or not there is competition among the spermatozoa derived from two sources. Crew (1926) mated White Leghorn hens both with White Leghorn cocks and with Redcap cocks and obtained 72 White Leghorn and only 17 crossbreds. Dunn (1927) mated White Leghorn hens with White Leghorn and Hamburg cocks and found that 90 per cent. of the offspring were sired by the White Leghorns. He also mated White Wyandotte hens alternately with White Wyandottes, Hamburgs and Pitgame cocks and obtained 92 chicks sired by the White Wyandotte and 15 by the others. Warren and Kilpatrick (1929) and Krallinger (1930) have confirmed Crew's finding that when two or more males are used in experiments of this kind it is usual for the latest male to inseminate the hens to claim most of the offspring. Bonnier and Trulsson (1938) have refined the experimental techniques employed in such investigations by substituting artificial for natural insemination. They have found evidence of competition between the spermatozoa from different cocks and of selective fertilisation even in cases when microscopical examination of the ejaculates failed to detect any appreciable difference between those derived from different sources. King (1929) double mated white female rats with white and with black males to get an excess of black offspring. Cole and Davies (1914) obtained similar results in the case of the rabbit, one of the two bucks claiming the majority of the offspring. When the sperm of this buck were alcoholised they could no longer compete with those of his rival, though it was shown that when present alone they could and did fertilise ova. In the case of the fowl and rat experiments it may be doubted whether equal numbers

of spermatozoa from each source were present in the mixture ; unless it is known that equal numbers of the two kinds of sperm are present, selective fertilisation cannot of course be established.

Hertwig (1906, 1907) by allowing frog's eggs to become "over-ripe" before fertilisation obtained from 70-90 per cent. of males among the resulting progeny, whilst Kuschakewitsch (1910), using the same methods, obtained males only. King (1910) subjected toad's eggs to dryness and thereafter obtained an excess of females. It is difficult to explain these results. If the female is homogametic, as would seem to be the case, such procedures may perhaps result in the extrusion of the X-chromosome from the egg of the frog so that subsequent fertilisation yields XO (male) individuals, or perhaps after such treatment the Y-bearing sperm is advantaged. If, on the other hand, the female is XY then maybe the X and the Y do not pass into the polar body equally often. But it is not improbable that the true explanation of these results is to be found in the more recent work on the factors concerned in sex-determination in the frog (Witschi, 1934, 1936, 1939 ; Burns, 1938). It is now established that the sexual fate of the individual, be it XX or XY in constitution, is determined by the developmental history of the cortical and medullary components of the embryonic gonad. In the normal female pursuing an uneventful history, the medulla disappears, whilst in the male the cortex does not develop. The decision as to which of these events shall occur is made by the relative strengths of the genetic male- and female-determining factors. But it has been shown experimentally that environmental conditions also influence the relative development of cortex and medulla. High temperatures, for example, encourage the development of the medulla and inhibit that of the cortex ; low temperatures have the opposite effect. Thus environmental factors by affecting in this way the differentiation of the gonad can disturb the sex-ratio, for under certain conditions genetic females become functional males and under others genetic males become females. Selective syngamy seems to be the explanation in the case of the hermaphrodite ascidian *Styela* (*Cynthia*) *partita* reported by Plough (1933).

In those forms in which the haplo-diploid sex-determining mechanism is present and in which fertilisation yields the diploid female and parthenogenetic development the haploid male, as is the case in the bee (Dzierdzon, 1845), the primary sex-ratio is, of course, determined by the relative frequency of fertilised and unfertilised eggs. In the bee the queen possesses and exercises the power of controlling the sex-ratio and the anatomical basis of this control is the sperm pump, by the use or non-use of which eggs can be fertilised or not.

The problem of controlling the primary sex-ratio in those forms with the homo-hetero-gametic mechanism and with male heterogamy, other than by incorporating "sex-ratio" genes into the genotype of the stock, is that of devising means whereby the gametes elaborated by the heterogametic male are required to pass a barrier of such a kind that one type of gamete (X-bearing or Y-bearing) is stopped or seriously handicapped whilst the other passes without harm or hindrance. The problem in the case of the heterogametic female is different ; it is that of disturbing the frequency with which the X and the Y chromosomes respectively pass into the polar body.

One method, now commonly associated with the name of Unterberger (1930), is that of increasing the alkalinity of the reproductive passages of the mammalian

females by means of sodium bicarbonate douches, the idea being that the Y-chromosome-bearing spermatozoon is advantaged in an alkaline environment. Douches of lactic acid, it is suggested, have the opposite effect, favouring the X-bearing gamete. The results which Unterberger obtained and the reasonableness of his argument attracted much attention, it being recognised that a more critical experimentation was demanded before his conclusions could be finally accepted.

Actually such experimentation has failed completely to substantiate Unterberger's claims. Schumacher (1934), for example, was unable to detect any correlation between the pH of the vaginal secretion and the sex of the offspring in a series of 1510 women. When he used 150 female mice and rats as experimental material he found that alkalinisation and acidification of the vagina resulted in complete sterility. Using rabbits, he found that in 26 litters produced after alkalinisation the sex-ratio was 81♂♂ : 75♀♀, and that in 25 litters following acidification with lactic acid there were 78♂♂ and 82♀♀. In a second series "alkalinised" litters gave 93♂♂ : 82♀♀ and "acidified" 95♂♂ : 95♀♀. Mahovka (1936), who surveys all the previous work on this subject, concludes that though the method of shifting the pH of the vaginal secretion is the one that deserves most attention, for the present it cannot be claimed that it has proved to be successful. (See also Hartman, 1939; Cook, 1940; McPhee and Eaton, 1942.)

Another method devised to produce the same result—the separation of the X and the Y spermatozoa—is that employed by Schroeder (1934). She presented the view that the two kinds of spermatozoa were positively and negatively charged respectively and therefore moved to the opposite poles of an electric field. Two groups of rabbits, totalling 58, were inseminated, one with the anode portion and the other with the cathode portion. The anode group included 30 per cent. females : 70 per cent. males; the cathode group 45 per cent. females and 55 per cent. males. In another series of some 60 rabbits the anode group gave 75 per cent. females, the cathode 68 per cent. of males. In both series the pH was 7.1–7.2. Schroeder thought that the difference in the results given by the two series was due to the fact that one experiment was carried out in summer and the other in winter, the temperature at which cataphoresis takes place being an important factor. In a third series, therefore, the temperature was controlled and cataphoresis carried out at 10°, 15°, and 25°. Fifty-one young were produced. At 10° the anode portion gave 83 per cent. females, at 15° 52 per cent. and at 25° only males were produced. Mahovka failed to confirm Schroeder's opinion that some spermatozoa are positively and others negatively charged, for all those examined proved to be negatively charged. Apparently their movement to different poles is due to the interaction of passive cataphoresis and active negative galvanotaxis.

Mahovka also investigated the effects of a high frequency field upon rabbit spermatozoa, using wavelengths of 3.68, 4.5 and 2.68 m. The most interesting results were obtained with the 3.68 m. The exposure varied in one series (A) from 6 to 18 minutes, in another (B) from 34 to 66 minutes. Female rabbits inseminated with this irradiated sperm produced 368 offspring, 112♂♂ : 256♀♀, i.e. 69.56 per cent. ♀♀ (controls 49.29 per cent. ♀♀). Since the proportion of females was about equal in both series (69.00 and 70.16 per cent.), the time of exposure would seem to be unimportant. It was found that the percentage of females was associated with the degree of inactivation of the sperm following irradiation, the fewer living sperm the greater the relative number of females. These results

suggest that there is a differential susceptibility on the part of the X-bearing spermatozoa to the factors acting in a condenser field—the Y-bearing being the more susceptible.

Now that the genetic and cytological evidence concerning the nature of the sex-determining mechanism has been reviewed, it becomes possible to assess the value of the many theories of sex-determination which have been based upon statistical studies of the secondary sex-ratio. The secondary sex-ratio is not in itself necessarily a true indication of the kind of sex-determining mechanism involved in any given case. Before it can claim much value it must be compared with the primary sex-ratio and any difference between the two must be explained. Furthermore, any theory based upon such studies must be in harmony with the demonstrated existence of the homo-hetero-gamety mechanism. Thus, for example, the hypothesis, of which there are many variants but which in this country is usually associated with the name of Rumley Dawson (1909) does not exhibit this harmony and is contradicted flatly by the results of critical experimentation; it can therefore be dismissed. It is simply not the case that in the mammals ova produced by the right ovum become males and those produced by the left become females. Nor is it the case that male-determining spermatozoa are derived from one testis, female-determining spermatozoa from the other (Doncaster and Marshall, 1910).

It has been observed that the secondary sex-ratio varies with the species, breed and strain, e.g. cattle (Gowen, 1942); sheep (Rassmussen, 1941). It varies with the season: greyhound (Dighton, 1922); horse, cattle, sheep, pig, dog (Wilckens, 1886; Heape, 1907); mouse (Parkes, 1924); rat (King, 1915). It varies also in different matings and in many cases the disturbance, as Jull (1923) has shown in the case of the fowl, would seem to be related to the physiological condition of the parents.

Data have been presented which sometimes support and at other times contradict the suggestion that the secondary sex-ratio is affected by the relative ages of the parents, the offspring being mostly of the same sex (or of the opposite sex) as the older (or as the younger) parent. Hofacker (1828) and Sadler's (1830) law—that the sex of the offspring is that of the older parent—finds no support in the result of critical inquiry; it is contradicted, for example, by the work of Schultze (1903) on mice. Some have advanced the view that the age of the mother has a relation to the sex of the offspring, younger mothers producing a preponderance of males (or of females). Other data have been presented to prove that the sex of the offspring tends to be that of the more (or of the less) "vigorous" parent. The theory of Starkweather (1883), for example, suggests that the "superior" parent tends to beget offspring of the opposite sex. There is no experimental basis for such theories as these. It must not be forgotten that in the majority of cases the data upon which these theories are based have not been dealt with by the biometrically expert and that their genuineness cannot be guaranteed. Moreover, it is as yet impossible to define "vigour" and "superiority" in accurate physiological terms, so that these theories are not suitable for serious scientific discussion.

It has been suggested that the secondary sex-ratio varies with the time of service during the oestrous period (Thury, 1863; Düsing, 1884; Pearl and Parshley, 1913). Further data collected by Pearl (1917), however, did not support the

suggestion that service early in the heat period resulted in a female calf, late in the heat period in a male. The suggestion that there is a relation between the secondary sex-ratio and the size of the litter in polytocous animals is not supported by the results of Wentworth (1914) in the case of the dog and the pig, of King (1915) in the rat, of Parker and Bullard (1913), of Machens (1915), and of Parkes, (1923) in the case of the pig. The secondary sex-ratio varies with the parity, with the chronological order of the pregnancy, for in the case of the human subject the dog and the mouse, it has many times been noted that there is a continuous drop in the sex-ratio at each succeeding pregnancy. In the case of the rabbit, it has been shown that the secondary sex-ratio is related to the chronological order of the mating. Hays (1921) obtained a sex-ratio of 56·33 per cent. males as a result of first matings, this falling away to one of 21·87 per cent. males in the case of the twentieth matings.

It is unfortunately the case that data relating to the sex-ratio sufficient to make a thoroughly comprehensive study possible exist only for human populations. However, there can be no doubt that in general, observations concerning the sex-ratio in the human subject and interpretations devised to explain them, can be applied to the problems of the sex-ratio in other animal forms. In the Registrar-General's *Annual Reports* it will be found that the secondary sex-ratio amongst newly born babies is around 105·6 : 100, whilst the sex-ratio for babies who die during the seventh-ninth months of intra-uterine life will be found to be significantly higher than this. It is revealed, furthermore, that there is an astonishing swing in the sex-ratio as the five-year age groups are passed under review. There is a numerical preponderance of males amongst the earlier age groups which gives place to a numerical equality of the sexes amongst the 15-19-year olds, and thereafter, as age group succeeds age group, there is a female numerical ascendancy which progressively increases until amongst the 85s and over there are twice as many females as males. Reference to the mortality tables make it clear that this swing in the sex-ratio from high to very low is the result of a sexually selective mortality, for at all ages more males are removed from the population by death. Amongst those aged 75 and over actually more females than males die for the reason that amongst the individuals who are 75 and over awaiting death there are far more females than males.

An abundance of other facts relating to the human sex-ratio exists in the literature that deals with this particular subject. The sex-ratio amongst abortuses is accepted as being higher than that amongst still-births, and there is sufficient reason for holding the view that as a rule the sex-ratio amongst abortuses of the earlier months of intra-uterine life is higher even than that amongst those of the later months. The secondary sex-ratio is influenced by urbanisation, being lower in county boroughs than in rural areas. It is affected by social upheavals. In all the countries directly engaged in the first World War the secondary sex-ratio was high immediately following the cessation of hostilities and higher immediately after than immediately before the war ; neutral countries affected commercially by the war experienced the same phenomenon, though not to the same extent. The secondary sex-ratio is highest amongst the first-born, and declines with increasing size of family in a curvilinear manner. It is influenced by migration, the migrants sometimes having a higher or a lower secondary sex-ratio than their relatives who remain behind. As a general rule it is lower amongst illegitimates than amongst

living children born in wedlock. It is lower amongst coloured people than amongst the whites amidst whom they live. It is affected by social status, being higher in the upper and middle classes and lower amongst the unskilled workers. In places and in periods where infant mortality is high the ratio of boy deaths to girl deaths is low, and where there is a low rate of infant mortality the death-rate of boys is relatively high. With the reduction of infant mortality that has occurred within recent years there has been a marked relative increase of boy deaths. The sex-ratio amongst infants dying during the first year of life varies with the age of the infants; for the very young it is high, but decreases during the year. (Holmes, 1926; Russell, 1936; Heape, 1909; Punnett, 1903.)

If these conclusions are accepted then for them there would seem to be only one possible explanation. In the case of the human species the male, in virtue of his maleness, must be less viable than the female, so that, under unfavourable circumstances, both pre-natally and post-natally, the male, because of this greater inherent fragility, suffers more easily and more severely than does the female, and is removed from the population by death in greater numbers. Since this selective elimination of the male occurs before as well as after birth it follows that since the secondary sex-ratio is 105 : 100 the primary sex-ratio must be higher than this, high enough, presumably, to allow for the pre-natal wastage that occurs.

Adopting this suggested explanation, every one of the facts stated above can be accommodated. Abortion is common. Incidence of abortion is higher during the earlier months of pregnancy. If, then, the conditions attending pregnancy are unfavourable to the embryo and foetus, foetal death is made more probable, and if the male foetus is inherently less viable than the female, more males than females will perish. Under such circumstances the secondary sex-ratio will be low. The sex-ratio amongst abortuses will be higher than amongst still-births for the reason that the differences in viability between male and female are greatest during the earlier stages of intra-uterine development. Abortion and still-birth are more common in urban than in rural populations and for this reason the secondary sex-ratio will be lower in county boroughs than in rural areas. The sex-ratio is highest amongst the first-born for the reason that the incidence of abortion and miscarriage is higher in large than in small families. The sex-ratio differs in different countries and in different social strata in one and the same country because amongst these there are different standards of living and of personal and public hygiene, so that abortion and miscarriage are commoner in one country and social stratum than in another. It is influenced by migration because the migrant moves from one environment to another, from a harsh to a generous or *vice versa*. It is lower amongst illegitimates than amongst legitimates for the reason that abortion and still-birth are commoner amongst the former. It is lower amongst coloured people than amongst their white neighbours when the standards of life of the two sections of the community differ markedly. Usually the standards of the coloured peoples are lower and for this reason amongst them abortion and miscarriage are more frequent. The relation between the primary and secondary sex-ratios is unaffected when infantile mortality generally is high, and disturbed when this is low for the reason that high infantile mortality is due to the action of death-dealing agencies of such potency as to overwhelm any difference in respect of viability on the part of male and female. Such diseases kill boys and girls without discrimination and since both sexes are removed

the sex-ratio is unaffected. Low infantile mortality implies relatively mild attacks of disease-provoking agencies which discriminate between male and female, leaving the female untouched and removing the weaker male to yield a low sex-ratio. The rise in the secondary sex-ratio that is associated with protracted war is possibly due to the raising of the marriage rate and thus to the relative increase in the number of first babies. Another factor which is thought to operate is the higher degree of physiological fitness on the part of wives, the lengthy absences of husbands resulting in a diminution in the frequency of intercourse and therefore in pregnancy, with opportunities of rest and repair.

It is suggested that all these variations in the secondary sex-ratio are reflections of differences in the incidence of abortion and miscarriage. A greater frequency of abortion implies a high male mortality and therefore a low sex-ratio amongst those who remain to be born alive.

Ciocco (1938), in a very thorough study of the vital statistics of the United States over two decades, finds no evidence to support the majority of these conclusions and the speculations based upon them. He found no significant difference between the secondary sex-ratio in urban and in rural populations and that the sex-ratio for illegitimate births was practically equal to that for legitimate births when colour differences were taken into account. His data yielded indirect evidence which seemed to indicate that neither war, nor economic upheaval, nor climatic and geographical difference, nor general mortality and infant mortality, nor variations in the still birth rate and in the still birth sex-ratio was causally related to fluctuations in the sex-ratio. On the other hand, he found that since 1930 there had been a slight decline in the sex-ratio, that the sex-ratio of coloured births was consistently and markedly lower than that of births to native white parents, that the sex-ratio decreased as the order of birth increased and that on the whole births to parents below thirty years of age had a consistently higher sex-ratio. He maintains that there has been far too much speculative rationalisation about differential foetal mortality and urges that intensive research should be undertaken to discover more exact facts about this postulated mortality.

Heape's (1907a, 1907b, 1909) views concerning the sex-determining mechanism, though they must now be regarded as faulty in respect of detail, are easily restated in terms that are in accord with modern theory. His arguments presented to explain his sex-ratio figures anticipate in a truly amazing way all the major discoveries concerning selective sexual mortality. He concluded that there were two kinds of ova and that extraneous forces affected the relative survival rate of the two kinds. The modern view is that such forces affect the relative survival rates of the two kinds of zygotes, males and females, resulting from fertilisation.

It is unfortunate that the figures relating to pre-natal and early post-natal death amongst animals other than man are but few. However, in all cases in which the figures for the sex-ratio of still births have been examined, they show that this is higher than those amongst live births. Thus Goehlert (1888) gives 106: 100 for still births and 96.5 for live births amongst horses. In cattle Lillie (1916) found the sex-ratio amongst still births to be 134: 100, whilst Jewell (1921) gives 123: 100, that for live births being 100-110: 100. Parkes (1925) found that in the pig the sex-ratio showed that there was an inverse correlation between male percentage and the stage of development amongst the foetuses examined and concluded that the primary sex-ratio in these animals must be about 160: 100.

Krizeneky (1935) gives the sex-ratio amongst still born pigs as 119 : 100, compared with 101·8 : 100 for live births. Crew (1937) gives figures which show that whilst the secondary sex-ratio amongst 2336 pigs was 104·9 : 100, it had fallen to 97·4 : 100 amongst the 1489 which remained alive at the time of weaning, the percentage loss amongst the males being 38·6, that amongst the females being 33·7. These figures suggest that the pre-natal mortality in the pig which discriminates against the male is continued at least until the time of weaning.

The secondary sex-ratio of the albino rat is accepted as being roughly 106 : 100, that amongst still born rats was found by King (1921) to be 129·3 : 100. In certain mouse stocks Parkes (1924) found evidence of a considerable pre-natal mortality, this being sexually selective, falling preponderantly on the male. Later observations of Parkes, however, did not support this conclusion and McDowell and Lord (1925), dealing of course with an entirely different mouse stock, found no evidence whatsoever for any continuous sexually selective elimination during gestation.

In the case of birds, which possess from the point of view of the present discussion a peculiar interest in that amongst them the male is the homogametic sex, there is but little information concerning the sex-ratio that has been derived from controlled experimentation. Darwin accepted the common view that in Nature there is a preponderance of males as an argument that supported his theory of sexual selection. Pelseener (1921) states that amongst birds there is an early excess of males which gives place later, owing to the shorter life and higher mortality of the males, to an ultimate numerical preponderance of females. Cole and Kirkpatrick (1915) found a secondary sex-ratio of 105 : 100 in the pigeon and noted no swing in the secondary sex-ratio with advancing age. Haig Thomas and Huxley (1927) found that in pheasant species crosses there was a predominance of males at hatching and a large excess of male deaths both before and after hatching, so that a secondary sex-ratio of 67·1 per cent. males became reduced to 50·1 per cent. in the adult. Mayr (1939) in a most valuable paper has collected and analysed the widely scattered figures for the sex-ratio among wild birds. This sex-ratio is, of course, that which obtains in a population consisting of individuals of very different ages ; it is the so-called tertiary sex-ratio. It varies widely in different species and in a general way is related to the particular reproductive habits of the species. He shows that it is most difficult in the majority of cases to arrive at any reasonable estimate of the primary sex-ratio but concludes that inequalities in the tertiary sex-ratio are commonly reflections of corresponding inequalities in the primary sex-ratio.

In the case of the fowl there is, as might be expected, an abundant literature concerning the sex-ratio and mortality, much of which has been summarised by Landauer and Landauer (1931) and by Byerley and Jull (1935). Landauer and Landauer express the opinion that if any differential mortality during embryonic development occurs it is the male that suffers more, that there is a slight excess of females at hatching and that more males than females die in the early weeks of post-natal life. Byerley and Jull, on the other hand, presented the view that if and when there is any differential mortality between the sexes more females than males die before hatching, that there is a slight preponderance of females amongst the live-born and that there was no evidence whatsoever of any differential post-natal mortality between the sexes. Crew (1938) found the sex-ratio amongst

8565 dead-in-shell was 51.03 per cent. males, whilst the secondary sex-ratio amongst 515,976 sex-linked chicks was 50.34 per cent. males, and that amongst 2,216,051 chicks sexed by the Japanese method was 51.38 per cent. males. All these ratios are very near normality and as far as they go seem to show that there is little if any sexually selective mortality in these stocks. Hays (1945) recognising that there was very considerable variability in the secondary sex-ratio of different females within a breed of poultry, examined the records of 39 pure bred Rhode Island Red females mated to pure bred Rhode Island Red males over a period of ten years. The record of every female in respect of fertility and hatchability was known. The sex of all chicks, save those who died (61) before this time, was noted at the eighth week. Among 870 chickens sexed, the sex-ratio was 49.7, a figure not significantly removed from equality.

Geiser (1924-25), reviewing the literature dealing with the sex-ratio in the fish, found that in populations taken at birth the sex-ratio was either equality or else that there was a slight excess of females, whereas in older populations the females were always more numerous.

In the case of insects there are abundant records relating to the sex-ratio amongst a great variety of species. Many of these are summarised by MacArthur and Baillie (1932) and they show very clearly indeed that in these forms it is the rule that there is a selective elimination of the male irrespective of whether he be homo- or hetero-gametic.

In view of the facts that in all species examined the male is, or would seem to be, less viable than the female, irrespective of his sex-chromosome constitution, and that this difference in viability is exhibited at all ages, it is difficult, if not impossible, to ascribe this differential mortality to the action of sex-linked lethal genes, as has been attempted by Lenz (1923), Gunther (1923), Geiser (1924-5), Huxley (1924), and Schirmer (1929), among others.

There is, of course, no doubt whatsoever concerning the existence and effects of such sex-linked recessive lethal genes which produce structural defects or functional derangements at an early stage in development of such a magnitude that continued life is rendered impossible. The first "lethal" gene of this kind was discovered as long ago as 1913 by Rawls. Dozens of them have since been identified in *Drosophila* by the usual genetic techniques and hundreds of them have been produced by X-ray treatment (Muller, 1928). The action of other genes of this kind is not so drastic and they are therefore classified as near-lethals, semi-lethals and poorly viable mutants, and by the use of them in breeding experiments any sex-ratio between 100 : 100 to 0 : 100 may be obtained. The hetero-gametic male is affected by these lethals for the simple reason that he has but a single X in which there is either the lethal or else its normal allele. Males with the lethal suffer. The homogametic female, on the other hand, having two Xs can have the lethal gene in one, but this can be neutralised by the normal allele in the other. A single dose kills the male, but two doses are required in the case of the female.

There are lethals which kill females only. Mohr and Sturtevant (1919) found such a gene in *D. funebris*. Redfield (1926) found a gene in the second chromosome of *D. melanogaster* which does not kill a female homozygous for it, but which affects her eggs in such a way that few are able to develop if fertilised by an X-bearing sperm.

There is no doubt that these sex-linked recessive lethal genes are responsible for some of the higher mortality of the heterogametic sex, both pre- and post-natally. There are other genetic differences between the sexes that are sex-limited and not sex-linked and which tend to lower the viability of the male. Levit (1935), for example, has presented a very considerable body of evidence which shows that in man many defects and derangements are more often and more completely expressed in the male than in the female, and that many others that have been regarded as sex-linked are, in fact, sex-limited, being expressed only in the male. He explains the greater manifestation of heterozygous genes, corresponding to defects, in the male on the ground that selection is less intense among males than among females, so that these genes tend to accumulate in the male. He is of the opinion that, in man, few of the male deaths at or before birth are due to sex-linked lethals.

Since heterogamety cannot be regarded as the reason for the lower viability of the male it has to be concluded that "maleness" itself, a state which physiologically, endocrinologically, differs markedly from the alternative state of "femaleness," is the real cause of the greater fragility of the male. It is established that the male, in all the species examined, has the higher metabolic rate, and it has been suggested that this renders him less resistant to unfavourable conditions and more prone to death. Riddle (1931) assumes that the genes exert their influence during development by establishing higher or lower oxidation rates, and in this way bravely attempts to link up genetics and developmental physiology. Be this as it may, it is the case that the problem of the differential viability of the sexes is one that belongs mainly to the territory of the physiologist.

The extraordinary disturbance of the (secondary) sex-ratio among the progeny of species and racial crosses in animals is not to be explained in this way, for in these the heterogametic condition itself would seem to be the cause of the numerical disparity between the sexes. Haldane (1922), having examined the available figures, was able to state that "when in the F_1 offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterogametic sex." This rule holds for mammals, insects, birds and moths, as well as for other forms. Sterility, rarity and absence can reasonably be regarded as graded effects of one and the same cause, and the cause common to all forms must surely be found in the heterogametic constitution. One of the mechanisms accounting to a large extent for Haldane's rule is now clear and may be summarised as follows. In two closely related species the same developmental process may, in the course of phylogenetic divergence, come to be based on different combinations of genes, or on the same combination of genes differently distributed among the chromosomes. When one of the chromosomes involved is the X, the hybrid of the heterogametic sex, which receives its single X from only one parent species, may be affected by the disruption of any one such combination. This situation has been proved actually to occur in hybrids between species of *Drosophila* (Dobzhansky, 1937; Pontecorvo, 1943a). Craft, in 1938, found that the secondary sex-ratio among mule foals sired by 98 different jacks was 44.28 ± 0.89 per cent. males, whilst that amongst 1263 horse foals was 52.52 ± 0.95 per cent. The sex-ratio among still born mule foals was equality, whilst that amongst still born horse twins was 77.5 per cent. males. The sex-ratio for the mule was 8.4 per cent. below that for the horse and the difference was statistically significant. The sex-ratio of hybrids

between *Bos indicus* and *Bos taurus* was 52.8 ± 0.69 per cent. males ; that amongst calves of *Bos indicus* 50.92 ± 3.36 per cent. and that of *Bos taurus* 51.52 per cent. Craft concludes that his figures support the view that if there is a disturbance of the sex-ratio resulting from hybridisation the difference is to be found in the hetero-gametic sex.

It is impossible not to be impressed by the astonishing differences in respect of the sex-ratio, secondary and tertiary, that are encountered as one passes from species to species, by the swing in the sex-ratio in one and the same species when the different age groups in the population are surveyed and by the precision of the mechanisms that are responsible for the establishment of the sex-ratio. But it is difficult in the present state of our knowledge to appreciate the significance of these differences and of this swing. However, it is reasonable to adopt the view that they are not unrelated to the reproductive requirements of the species. A species grows, expands, spreads whenever external conditions are propitious. The major task of the individuals of one generation is the economical and efficient production of that number of functional gametes which will ensure that the number of individuals of the succeeding generation will be in harmony with the resources of the environment. If there can be an optimum population size there must be an optimum gamete number. The females of the parental generation are required to produce this optimum number of ova ; the males are required to produce that number of spermatozoa which will make the fertilisation of every one of these ova highly probable. The number of females, therefore, must be related to, among other things, the number of ova one female can elaborate in a given time. The number of males must be related to the number of spermatozoa one male can elaborate in a given time, to the variety of the fertilisation process, to the pre- and post-natal relationship of female and offspring, to the relationship of male and female in respect of courtship and parentage and to many other factors.

What would seem to be of major importance is that there should be a sufficient number of males to ensure the efficient fertilisation of every available ovum. It is possible, therefore, to think of a "reproductive" sex-ratio, an "optimum" sex-ratio among those individuals in a population of the ages which are associated with the expression of the reproductive function. Obviously this optimum must vary widely from species to species, from time to time and from place to place. If the male is merely a fertilising agent then he may be serving the interests of the species best by dying *in coitu*, since by so doing saving in food energy might be effected. On the other hand, if the male plays a prominent rôle in the pre- or post-natal care of the young, it might be advantageous to the species if the sex-ratio were equality or if adult males should outnumber the females.

This "reproductive" sex-ratio should be that which obtains among those individuals in a population of the ages that fall within the reproductive phase of the life history. In a human population the age group 15-19 includes those who stand at the threshold of their reproductive career. If mating is a biological affair, as opposed to marriage which is a social custom, and if pair-mating, during the evolution of man, has attached to itself a definite and positive value, it might be expected that there should be a sex-ratio of near equality among the 15-19 year olds. It will be found that in the Registrar-General's *Annual Reports* the sex-ratio among the 15-19 year olds is nearer equality than is that of any other age group ; among them alone are there equal numbers of males and females.

If this notion of an optimum reproductive sex-ratio is warranted, then it is reasonable to examine once more those phenomena which are concerned in its establishment—mutation, sex, heterogamy and differential mortality.

Mutation is the mechanism which provides the material possibilities of evolutionary change. A gene that has been tested and proved worthy, that has come to be in harmony with the rest of the genotype and through this with the external world, mutates and is thus replaced by another, its allelomorph, the merits of which have yet to be assessed. Mutation implies a disturbance of an equilibrium within the genotype itself and between this and the environment. It is usual, therefore, to find that mutation leaves the genotype less in harmony with the existing external conditions than it was before. Since the effect of any gene is influenced more or less profoundly by the action of all the other genes in the same genotype, it follows that if a new mutant gene is to be thoroughly tested it should become incorporated into as many different genotypes as possible as quickly as possible, since in one or more it may prove to bestow an advantage on the individuals possessing it.

Sex is the mechanism that has evolved and become adapted for the rapid dispersal of such a mutant gene among a population. A mutant recessive autosomal gene, and the majority of mutations are recessive, can be quickly incorporated into a variety of genotypes and can pursue its own evolutionary development before the character corresponding to it is expressed. The great usefulness of sex lies in the fact that it provides for variety in gene recombination and for the exercise of selection. Its advantages will be best exploited when among those about to reproduce there is a sex-ratio of equality.

The homo-hetero-gametic mechanism is the device that has become adapted for the efficient production of a primary sex-ratio of equality. In its evolution it has taken the form of the replacement in one sex of an X-chromosome by a partially or largely non-homologous Y-chromosome (cf. Fisher, 1931). An indirect result of this has been that a recessive mutant gene in the differential segment of the X in the XY individual is at once uncovered and so, if its action is deleterious or lethal to it no time is allowed for the finding of modifying gene companions and for the pursuit of its own evolutionary development. It is expressed and tested within a very short time of its first appearance and should it cripple or kill it is the heterogametic sex that is affected. In this way the secondary sex-ratio is disturbed.

But the greater fragility of the male, resulting in a differential mortality both pre- and post-natally, tends to disturb the results produced by the homo-hetero-gametic mechanism or to make the primary sex-ratio of equality unsatisfactory. To compensate for this, accessory devices have evolved. These are such as to lead to the unequal production of the two sorts of gametes, e.g. sex-ratio genes, (Sturtevant and Dobzhansky), selective syngamy (Plough), preferential orientation of the sex-chromosomes on the spindle (Vandel).

If these suggestions are warrantable, it follows that it is appropriate that a geneticist should take part in the making of this book. The meaning of sexual reproduction would seem to be revealed by genetical exploration.

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CHAPTER 23

THE LIFE-CYCLE

By JOHN HAMMOND AND F. H. A. MARSHALL

Reproduction in its widest sense is not complete until the mature size and form of the animal is arrived at. The union of sperm and ovum is only the beginning of this process and broadly speaking the growth of the individual which proceeds from this takes place first within the egg-shell (birds and most fish and reptiles) or within the uterus (mammals¹) and later independently of the parent, although for a varying length of time the young may have to depend for nourishment either wholly (suckling period) or in part on the parents. The stage of development at which the young are hatched or born varies greatly in different species and determines the extent to which parental care is necessary. Thus in the rabbit the young are born blind and naked and require a warm nest to be made for them by the mother, whereas in the guinea-pig the young are born in an advanced state and can fend for themselves in a few days. The extent to which "family life" is experienced in different species, therefore, varies widely, the duration of this in broad terms extending up to the time when the physical and mental characteristics of the species have been reproduced. The intimate relationship between these phases of the mammal's life—reproduction proper, the suckling period with its maternal instinctive relations between parent and offspring, and the period of growth and development of the full faculties, finds its counterpart in the close relationship of the origin of the internal secretions controlling them—the gonadotrophic, lactogenic and growth hormones of the anterior pituitary gland respectively, each in its turn occupying a dominant phase in the life of the individual. Details concerning the period of the life span which is spent in gestation, to puberty, and in growth for several species of animals are given in Table I (from Asdell, 1946).

As has been said by Verworn (1899) there is an essential similarity between reproduction and growth, both processes involving an increase of living substance, the difference between them consisting only in the fact that in the latter the newly formed substance remains in constant connection with the original organism and increases its volume, whereas in the former it is in most cases set entirely free: in the Protozoa, however, there are various transitional stages between these two.

Growth, like reproduction, involves cell division. As the mass of living substance increases, the cells must multiply, for every cell has assigned to it a limit in size beyond which it cannot pass. This is because the exchange of materials required for life takes place only on the surface of the cell, and as cell

¹ The monotremes (*Ornithorhynchus* and *Echidna*) are exceptional among mammals, laying eggs like birds.

TABLE I

RELATION OF GESTATION, PUBERTY AND GROWTH TO LIFE SPAN—FROM ASDELL (1946).

Species		Length of Gestation, Months	Age, Conception to Puberty, Months	Length of Growth Period from Conception, Months	Life Span from Conception, Months	Percentage of Life Span		
						To Birth	To Puberty	Spent in Growth
<i>Arvicola amphibius</i>	Water vole	7	5.7		16.7	4.0	34	
<i>Sciurus vulgaris</i>	European red squirrel	1	12		21	5.0	57	
<i>Cricetus auratus</i>	Golden hamster	5	2.5	6.5	24.5	2.0	10	26
<i>Microtus agrestis</i>	Field vole	7	1.7		24.7	2.7	7	
<i>Mus musculus</i>	Mouse	7	2.2	7	28	2.4	9	25
<i>Rattus rattus</i>	Rat	7	2.7	7.3	30.7	2.2	9	24
<i>Erinaceus europaeus</i>	European hedgehog	1.3	13.3		31.3	4.2	42	
<i>Onychomys leucogaster</i>	Grasshopper mouse	1	4		35	2.9	11	
<i>Clethrionomys glareolus</i>	Bank vole	7	5.7		36.7	2.0	15	
<i>Mustela erminea</i>	Ermine	2	14		41	5.0	34	
<i>Cavia porcellus</i>	Guinea pig	2	4	13.3	50	4.0	8	26
<i>Peromyscus maniculatus</i>	Deer mouse	8	2.4		57	1.4	4.2	
<i>Myocaster coypu</i>	Nutria	4.5	12.5		60.5	7.4	21	
<i>Oryctolagus cuniculus</i>	Rabbit	1	8	11	61	1.6	13	18
<i>Mustela putorius</i>	Stoat	1.5	13.5		61.5	2.4	22	
<i>Ondatra zibethicus</i>	Muskrat	1	13		71	1.4	18	
<i>Trichosurus vulpecula</i>	Australian opossum	5	12.5		72.5	7	17	
<i>Cynomys leucurus</i>	Prairie dog	1	13		91	1.1	14	
<i>Rucervus eldi</i>	Thameng	6	24		96	6.3	25	
<i>Sciurus carolinensis</i>	Grey squirrel	1.5	13.5		97.5	1.6	14	
<i>Vulpes vulpes</i>	Red fox	2	12		98	2.0	12	
<i>Marmota monax</i>	Woodchuck	1	13		101	1.0	13	
<i>Castor canadensis</i>	Beaver	4	28		104	3.9	27	
<i>Martes caurina</i>	Pine marten	8	23		104	7.7	22	
<i>Procyon lotor</i>	Raccoon	2	14		110	1.8	13	
<i>Odocoileus hemionus</i>	Mule deer	5	53 (!)		113	4.4	47	
<i>Canis familiaris</i>	Dog	2	9		122	1.6	7.5	
<i>Canis latrans</i>	Coyote	2	26		122	1.6	21	
<i>Vulpes fulva</i>	Red fox	2	12		122	1.6	10	
<i>Sus scrofa</i>	Pig	4	8	12	124	3.2	6.4	10
<i>Hapale jacchus</i>	Marmoset	5	19		125	4.0	15	
<i>Rangifer tarandus</i>	Reindeer	7.5	25.5		127.5	6.0	20	
<i>Phoca vitulina</i>	Harbor seal	10	34		130	7.7	26	
<i>Martes pennanti</i>	Fisher	11	23		134	9.0	17	
<i>Canis lupus</i>	Wolf	2	26		134	1.5	20	
<i>Capra hircus</i>	Goat	5	12	20	149	3.4	9	14
<i>Ovis aries</i>	Sheep	5	12	20	150	3.3	8	13
<i>Felis catus</i>	Cat	2	17		170	1.2	10	
<i>Macaca mulatta</i>	Rhesus monkey	5.3	41.3	57.3	185	2.9	22	31
<i>Otaria jubata</i>	Sea lion	11	83		211	5.2	40	
<i>Bos grunniens</i>	Yak	8	32		218	3.6	15	
<i>Bos taurus</i>	Cattle	9	15	56	225	4.0	6.5	20
<i>Euarctos americanus</i>	Black bear	7	43		247	2.8	17	
<i>Equus asinus</i>	Donkey	12	24		252	4.8	10	
<i>Ursus arctos</i>	Brown bear	7	79		287	2.4	27	
<i>Equus caballus</i>	Horse	11	23	60	311	3.5	7.5	19
<i>Rhinoceros sumatrensis</i>	Two-horned rhinoceros	7	247 (!)		300	2.3	82	
<i>Rhinoceros simus</i>	White rhinoceros	18	72		498	3.6	14	
<i>Elephas maximus</i>	Indian elephant	21	177		501	4.2	35	
<i>Homo sapiens</i>	Man	9	153	249	849	1.1	18	30

size increases the proportion of surface to volume diminishes. Cell division goes on, though with gradually decreasing frequency, throughout practically the whole of life. Tissue formation continues, but from an early period of development onwards there is a progressive diminution in the power of growth, this decline occurring at different rates in different tissues and parts in the body, thus giving rise to changes in the form of animals as they develop (*see* p. 817).

Increase in the number of cells, is, however, specially characteristic of the embryonic period. In the later stages of development growth occurs in a great measure through cell enlargement and the deposition of intracellular substance. The conclusion of this stage sees the animal in the prime of life in full reproductive activity with complete physical and mental development. This stage is followed

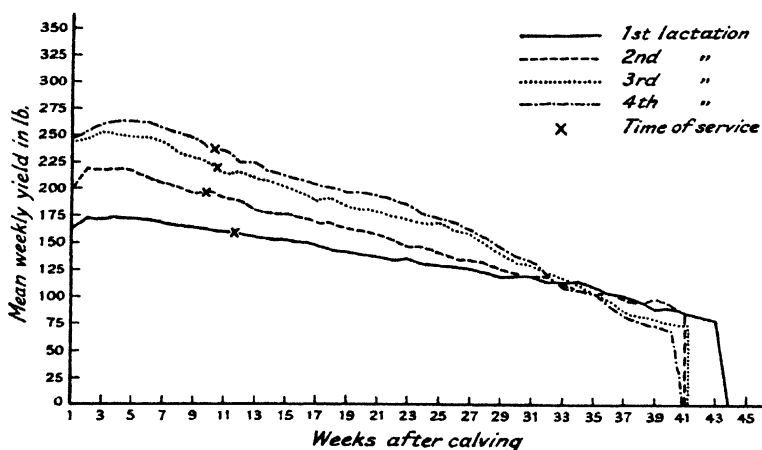


Fig. 23. 1—Curves showing the effect of age on the weekly yield of milk during the lactation period in cows. With increase in age up to the 4th lactation at about 7 years old there is increase in the initial yield, but as the cow becomes older the decline at the end of the lactation curve is more rapid. 1st lactation ———; 2nd lactation - - - - -; 3rd lactation ······; 4th lactation - · - · - ·. Time of service X. (From Sanders, 1928.)

by one in which the accumulation of intracellular substance outstrips the formation of new tissue and senescence gradually sets in until one by one the various tissues of the body begin to wear out. For example, an organ such as the udder of the cow which has many life-cycles during the cow's life is stimulated to growth during pregnancy, reaches its maximum activity some six weeks after calving, and thereafter begins to lose its activity and gradually passes into senescence. The body as a whole goes through a similar process, and this can be traced in the shapes of the lactation curves at different periods of the cow's life (Sanders, 1928). In early life the organ is in the rudimentary and non-functional stage; with the first pregnancy the cell growth does not reach its full activity and so the maximum weekly milk yield is small, but the cells retain their activity for a long time since their senescence in the young animal is slow; at maturity (4th lactation) full development of the cells is reached, and so the maximum daily yield is large (*see* Fig. 23. 1); while in old age not only is the extent to which the cells will grow reduced,

but the onset of their senescence is rapid. This life-cycle of one organ superimposed as it is on the life cycle of the animal as a whole gives us a picture of the changes occurring in the physiological processes of the animal during the different phases of its life. Many such age changes in physiological processes have been quoted by Brody (1927e, 1945). The rise and decline in the strength of grip with increasing age in man is shown in Fig. 23. 2.

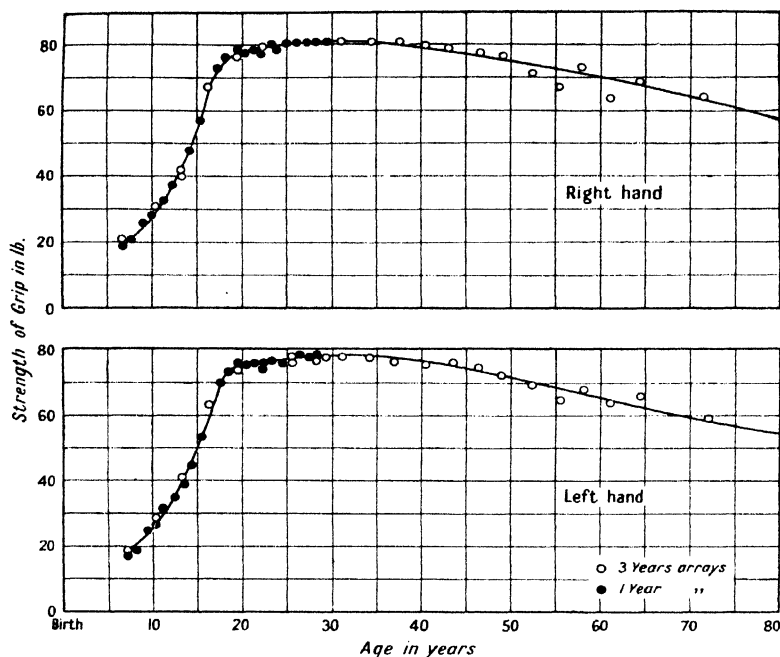


Fig. 23. 2—Curves showing the effect of age on the strength of grip in man. The curve rises rapidly up to a maximum between 20 and 30 years old and then slowly diminishes in old age. (From Brody, 1927e.)

I. GROWTH

Minot (1907) has compared the growth of the body to a man building a wall.

“He begins at first with great energy, full of vigour; the wall goes up rapidly: and as the labour continues fatigue comes into play. Moreover, the wall grows higher, and it takes more effort and time to carry the material up to the top of the wall, and to continue to raise its height, and so, as the wall grows higher and higher, it grows more slowly and ever more slowly, because the obstacles to be overcome have increased with the very height of the wall itself. So it seems with the increase of the organism, and with increase of development, the obstacles to our growth increase.”

According to Minot, the explanation of this phenomenon must be sought in the differentiation of the protoplasm which goes on growing with an ever-increasing complexity as the cells of the body multiply.

It has previously been mentioned that every cell has assigned to it a definite limit in size beyond which it cannot go. Boveri (1905) enunciated the general

law that the process of cell division is regulated by the proportion of chromatin material to cytoplasm, and that it comes to a standstill when the ratio of the mass of chromosome to that of the cell-substance in any given tissue or organ reaches a certain definite point. Furthermore, it is stated that the size of the cells in any given tissue after active cell multiplication has ceased, bears a definite relation to the original mass of chromatin contained in the fertilised egg (Robertson, 1908, 1913; Feldman, 1920). Thus it is pointed out that the mesenchyme cells

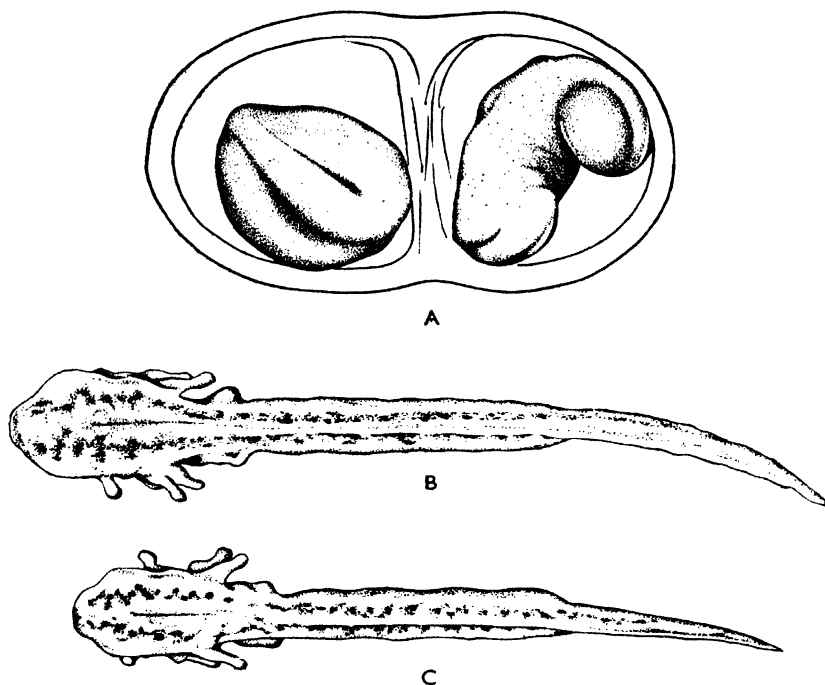


Fig. 23. 3—A. Two embryos from fragments of the same egg, one of which is haploid and the other diploid. B. Later stage of diploid larva. C. Later stage of haploid larva. (From Morgan, 1927, after Spemann.)

of the embryo developed from the artificially fertilised sea-urchin's egg are only half the size of those of the embryo which had been produced by normal fertilisation, for although the parthenogenetic and normally fertilised eggs are equal in size at the commencement of segmentation, the latter possess initially twice as much nuclear substance as the former (Driesch, 1908; Morgan, 1927). Similar dwarf embryos result from development proceeding from egg fragments (Fig. 23. 3). Conversely the union of two fertilised ova in rats after removal of the zona pellucida has produced a single oversized foetus (Nicholas and Hall, 1942).

The fact that cell division ceases when the mass of chromosomes in the nucleus of an egg (or of a tissue or organ) to that of the surrounding protoplasm reaches a certain definite limit, is regarded by Loeb (1906a) as evidence that this ratio is determined by the laws of mass action and chemical equilibrium. He says further

that if this conclusion is correct the synthesis of nuclear compounds, from their protoplasmic constituents, must be a reversible process.

The fertilisation of an ovum is immediately succeeded by an enormous synthesis of nuclear material. In the cellular division which follows, each new nucleus is of the same size as the parent nucleus. From this fact Loeb (1906b, 1907) concludes that the nucleus itself, or one of its constituents, acts as a catalyser

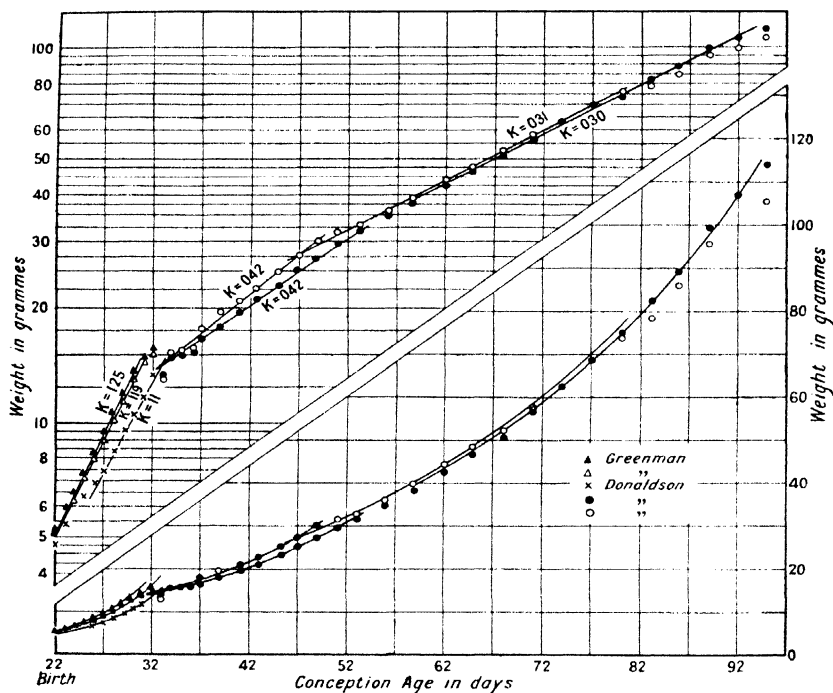


Fig. 23. 4—Curves showing "growth cycles." Growth data of the rat plotted on arithlog paper beginning with birth (about 22 days after conception). There appears to be a drop in the percentage rate of growth ten days after birth (about 5 per cent. per day) and possibly another about 1 month after birth (about 3 per cent. per day). Puberty occurs about 95 days after conception when the self-accelerating phase of growth is changed to the self-inhibiting phase of growth. (From Robbins, *et al.*, 1928.)

in the synthesis of nuclein in the fertilised ovum. Robertson (1908, 1913) quoting partly from Loeb, writes as follows :

" If the mass of the original fertilisation nucleus be m , the mass of nuclear material increases during the first segmentation period to $2m$, during the next to $4m$, and so on in geometrical progression. The duration of the various periods of segmentation, however, matters very little. Hence in the first unit of time after the beginning of cell division, a mass m of nuclear material is formed, in the second a mass $2m$, in the third a mass $4m$, and so on ; thus the velocity of the synthesis *increases* with lapse of time and with the mass of nuclear material already formed. This is a characteristic of that class of reactions known as autocatalytic, in which one of the products of the reaction, or, in this case, one of the constituents of the nucleus, accelerates the reaction. During the process outlined above, an emphatic disproportion between nuclear and protoplasmic

material has been established. As the nuclear synthesis becomes slower, however, the disproportion tends to adjust itself until, finally, the growth of the organism consists almost entirely of the growth of protoplasmic material, and culminates in the final re-establishment of the equilibrium between cytoplasm and nuclear material."

By the construction of mathematical curves to express these reactions Robertson (1923) has investigated the quantitative relations which exist between the amount of growth and time of growth. Brody and his collaborators (1926-1927a and b) have similarly worked out mathematical curves (Fig. 23. 4) and equations to express the normal growth rate occurring not only in the different species of farm and laboratory animals but also those in different breeds.

Robertson (1913) concluded that there are two or more growth cycles representing autocatalytic processes which make up the total growth of an individual. In man there are three maxima of rate of growth representing three phases or growth cycles. One of these is intra-uterine, but it is probable that this is not quite complete at birth. The second growth cycle seems to attain its maximum annual increment at about the fifth year, since the increment in weight at that age, as deduced from an investigation on growth in English boys, considerably exceeds the annual increments for the years immediately following. A third maximum in yearly increments occurs in males at about the sixteenth year, that is, at about the time of puberty. He suggests that the first growth cycle in mammals represents the course of the autocatalytic synthesis of the nuclear substance, that the third cycle represents the period during which cytoplasmic material is built up, while the second growth cycle is intermediate, representing a time when both synthetic processes go on contemporaneously. Many other workers have discovered such growth cycles in different species of animals. In the rat, according to Donaldson (1906, 1915) there are two intra-uterine growth cycles, while there is only a single well-defined extra-uterine cycle. Brody (1921) in the fowl, Brody and Ragsdale (1921) in cows, Murray (1921) in cattle, Fox, Trowbridge and Hogan (1923) in sheep, and others have found evidence of growth cycles in post-natal life, while Read (1913) in guinea-pigs, and Kislovsky and Larchin (1931) in cattle, among others have all described intra-uterine growth cycles. Hammond (1932) has questioned the constancy of many of the growth cycles in that nutritional influences, such as the amount of milk supply available for the growing lamb, can alter the time at which the maximum rate of growth occurs (*see* Fig. 23. 5). Thus in breeds which have a plentiful milk supply (Suffolk) the maximum rate of growth in single lambs is made immediately after birth, whereas when the milk supply is shared between twins the maximum rate does not occur until the third month, and between triplets not until the fourth month of life. In other breeds with a poor milk supply (Merino-Shropshire) even single lambs do not attain their maximum growth rate until the second month of life. Mitchell, Card and Hamilton (1926) also concluded that the apparent growth cycles in chicks were due to environmental conditions.

On the grounds that other factors such as food and temperature are of much more importance than age in determining the size of fishes, Kellicott (1908) criticises the autocatalytic theory of growth as applied to fish. While in birds and mammals growth is determinate and there is an average mature adult size for the species, in fish growth is indeterminate and there is no set adult size (Hecht, 1916).

Related possibly to this indeterminate growth in fish as compared with mammals there is the fact that as the fish grows up there is little or no change in the proportions of its body as compared with the extent to which this occurs

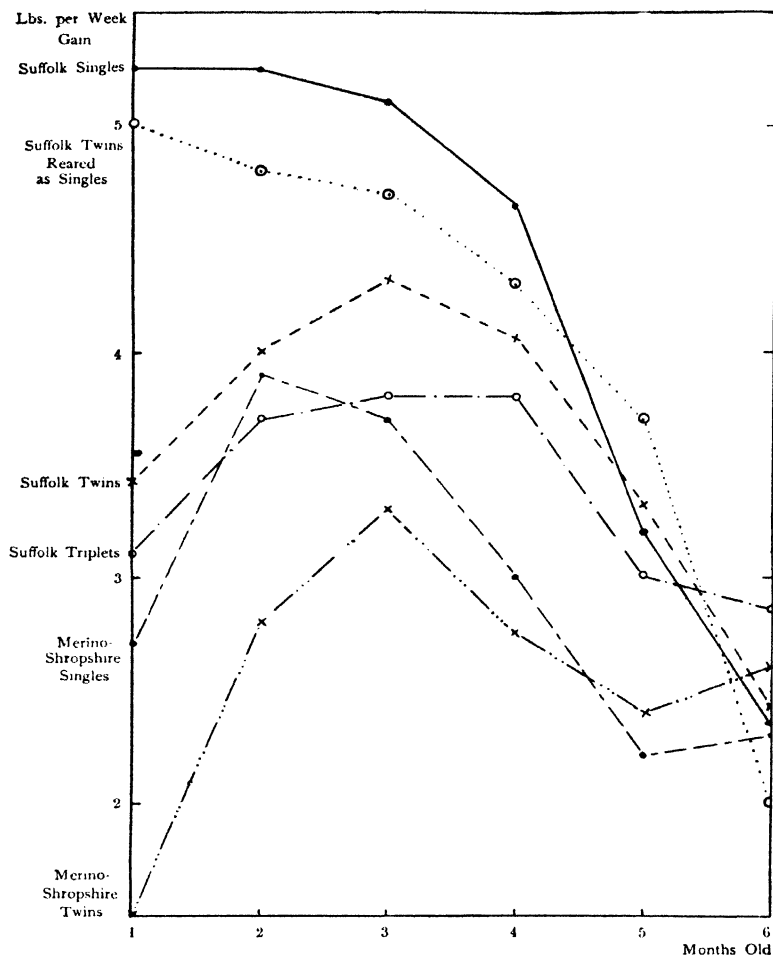


Fig. 23. 5—The rate of growth of single, twin reared as single, twin and triplet Suffolk lambs. The curves show how the amount of the milk supply determines the shape of the growth curve: the maximum growth rate of singles is made during the first month of life, while in the twins, which have to share the milk supply of the ewe, the maximum growth rate is not attained until the third month. The Merino-Shropshires do not milk so well as the Suffolks and so the single lambs of this type do not make their maximum growth rate until the second month of life. (From Hammond, 1932.)

in mammals (Hecht, 1913, 1916). There is also some evidence that this occurs in frogs (Donaldson and Schoemaker, 1900). The differentiation and specialisation of different parts of the body in mammals leads to a differential growth rate in the different tissues and parts of the body and so the proportions change as the

animal grows up (*see* p. 817); such changes are probably concerned in the limitations of size imposed on mammals as compared with fishes. The power of the different tissues of the body in mammals to sustain growth has been discussed by Smith (1932).

Pre-natal Growth

Many different ways of measuring growth have been used by different investigators. These vary mainly according to the point of view from which growth has been studied. Workers engaged in studying the various theories of growth (Minot, 1907) have usually used the percentage increment method (*see* Fig. 23. 6); whereas those who are interested in the various practical applications of growth studies have used either the actual growth curves (*see* Fig. 23. 7) or on the actual increments per unit of time (*see* Fig. 23. 5).

From the point of view of growth theories, for example, Minot (1907) found that the rate of growth in the rabbit-embryo (as measured by percentage increments) decreased from the 9th to 15th day, when the rabbit adds on the average 704 per cent. to its weight daily, to the 15th-20th day, when the average daily addition is only 212 per cent. He estimates that over 98 per cent. of the original power of growth in the rabbit or chick has been lost at the time of birth or hatching and that a similar fact is equally true of man (*see* Fig. 23. 6). "We start out at birth certainly with less than 2 per cent. of the original growth power with which we were endowed. Over 98 per cent. of the loss is accomplished before birth—less than 2 per cent after birth."

In contrast, where the object of the study of growth has been to determine nutrients required per day by the developing embryo, etc., growth has been measured (*see* Fig. 23. 5) on the grs. gained per day basis (Hammond, 1935b) or on curves of the actual weight attained (Hammond, 1937), and quite a different picture of the growth is obtained. Measured in this way the growth of the rabbit embryo itself is small as compared with that of the placenta and foetal fluids in the early stages of pregnancy (*see* Fig. 23. 7), whereas after about the 20th day it increases at a greater rate than any other part of the uterine contents.

Each tissue and part of the developing uterine contents has an individuality of its own in respect of the time at which the maximum growth occurs—some

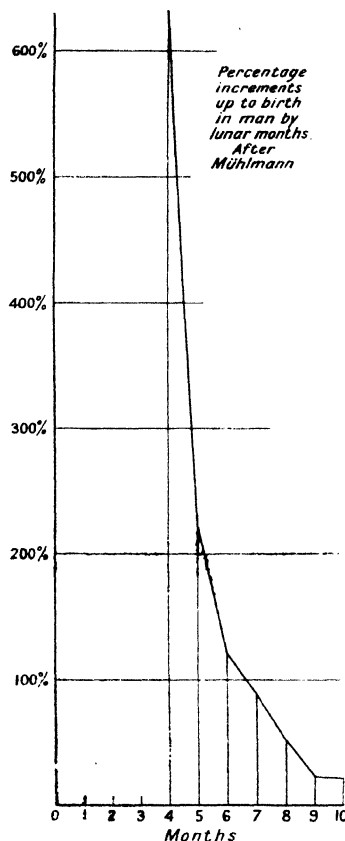


Fig. 23. 6—The percentage rate of growth in man before birth. The time intervals correspond to the ten lunar months of gestation. The rate of increase in the first three months is not indicated, since there are no statistical data on which to found any knowledge. From the third to the fourth month the increase in growth is 600 per cent., after which it quickly drops until during the last month of pregnancy it is barely 20 per cent. (From Minot, 1907.)

develop early and some late. In the rabbit the maternal placenta reaches its maximum growth rate earliest—at about the 14th day, after which time it fails to make much further growth. The foetal placenta and membranes form the organ of nutrition of the embryo and so develop early in life, being heavier than the embryo itself up to about 18 days of pregnancy; thereafter, however, although the foetal placenta continues to grow throughout pregnancy the rate of its growth falls far below that of the embryo itself. The foetal fluids also develop early, for one of their functions is to expand the uterus for the developing embryo, which later grows and replaces them (*see further* Chapter 16).

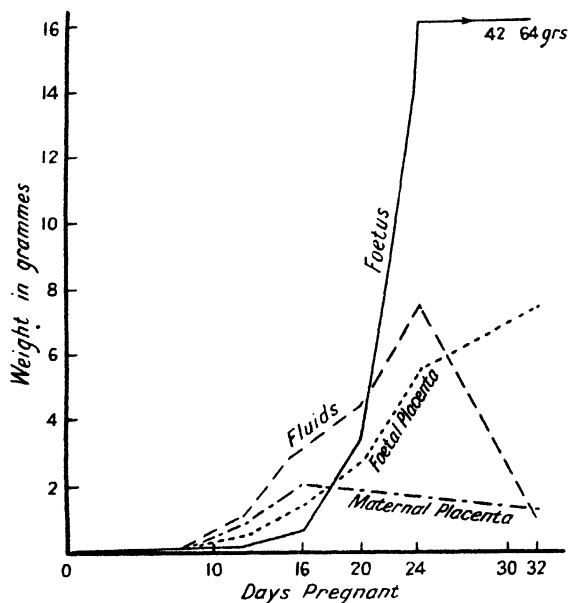


Fig. 23. 7—Growth of the various parts of the uterine contents of the rabbit during pregnancy. Up to the 16th day most of the growth made consists of maternal and foetal placenta and foetal fluids. During the second half of pregnancy, however, the foetus itself grows at an ever increasing rate while the actual weight of the foetal fluids declines greatly and that of the maternal placenta slightly. (From Hammond, 1937.)

It is well known that the birth weights of individuals which are members of a large litter are smaller than those of small litters (King, 1935). Thus single lambs may be up to 120 per cent. and triplets only 90 per cent. of the weight of twin lambs at birth (*see references in* Hammond, 1932). In the case of rabbits, where the variation in the size of the litter is larger, there may be very considerable differences in the weight of the young at birth due to this cause. Thus within one strain Hammond (1934) found the average weight of the young at birth to vary from 44 grs. in litters of 13 to 98 grs. in litters of 1 (Fig. 23.8). The smaller litters, however, usually have the longer duration of pregnancy, but it has been calculated (Wishart and Hammond, 1933) that the decrease of birth weight is from $1\frac{1}{2}$ to $2\frac{1}{2}$ grs. per young for each additional animal in the litter

independent of decreases due to the shortened length of pregnancy in the large litters (*see* Fig. 23. 9). The inhibition of the growth rate in individuals of large litters becomes much more marked as pregnancy proceeds; for example Ibsen (1928) found that in guinea-pigs the differences were not apparent until the 55th-day stage is reached. In rabbits (Hammond, 1935b) there is from the 24th day onwards a distinct and ever increasing difference in the rate of growth of the foetuses in small as compared with large litters; before this time differences, however, are shown in the foetal placenta size although not in the foetus itself. Ibsen's (1928) results show that this is also true for guinea-pigs.

It might be argued that the inhibition of growth in the young of large litters

is due to crowding in the uterus and so to defective nutrition through lack of space ; but this is not so, for if one ovary be removed and all the young are produced in one horn in the rabbit's uterus instead of being distributed between two, the pregnant uterine horn will enlarge much beyond its normal size but the weight of the individual young will not be materially affected. In any one doe, too, the weights of the young in a uterine horn containing a few young are not appreciably larger than in a horn containing many young (Hammond, 1935b). The inhibition of growth in the foetuses of large litters, therefore, is not due to the large number present in the uterine horn, but to limitation of some nutritional substance in the blood supply of the mother as a whole. That superabundance of energy-nutrition cannot increase the weight of the young beyond the normal limits imposed by other factors is suggested by Kopeck's (1924) finding that the weight of the newborn rabbit has no significant correlation with the quantity of stored material amassed in the pregnant mother. It would appear rather that the limitation in the amount of incoming nutrients or of some special growth substance or metabolic product of the mother is involved (Wishart and Hammond, 1933). Snyder (1934) has shown that by injecting anterior pituitary extracts into the pregnant doe the size of the young at birth is greatly increased, although the pregnancy is prolonged ; for example, a litter of 14 young rabbits born on the 38th day of pregnancy averaged 71 grs. each at birth (see Fig. 23. 10).

Gregory and Castle (1931) have shown that breed differences in the size of the rabbit foetus are distinguishable in the early stages (41 hours and subsequently) by differences in the number of blastomeres formed ; and they have suggested (Gregory and Goss, 1933a, b, c) that different amounts of a special substance of the sulphhydryl group in the embryo (glutathione) is the cause of these size differences. It may well be that the amount of this substance (or its precursors) supplied by the mother is the limiting factor retarding the growth of the young. The offspring from young mothers which are themselves still growing are notably smaller than from mothers which are adult (Eckles, 1919 ; Hammond, 1932). In horses it has been shown (Walton and Hammond, 1938) that the size of the young

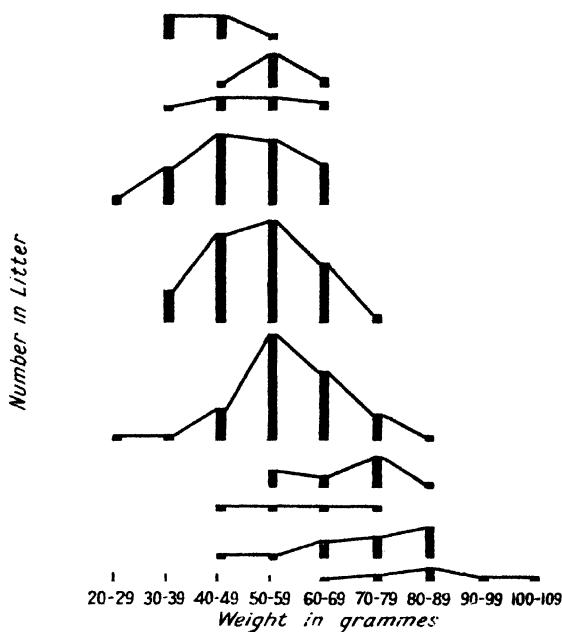


Fig. 23. 8—Variation in birth weights of individual young rabbits from litters of different sizes, born on 33rd day after conception. The height of the dark line represents numbers of young falling in the weight group. As the size of the litter decreases the weight of the individual young gradually increases. (From Hammond, 1934.)

at birth is greatly dependent on the size of the mother ; in reciprocal crosses between the large Shire horse and the small Shetland pony the size of the foal at

Number in Litter

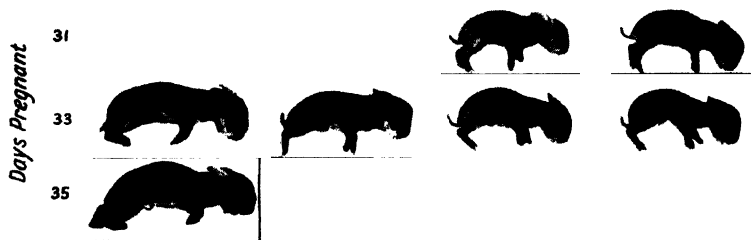


Fig. 23. 9—New born rabbits of an inbred strain to show how the number in the litter and the duration of pregnancy affect the body weight and stage of development reached at birth ; the latter is shown by differences in body proportions and hair growth. The weights of the young shown vary from 98 grs. from litters of one born at 35 days to 44 grs. from litters of eleven born at 31 days. (From Wishart and Hammond, 1933.)



Fig. 23. 10—Increased birth size in rabbits brought about by prolonging pregnancy by injections of anterior pituitary substance which produces new and active lutein tissue. On the right is shown the normal new born rabbit : on the left is a larger and more mature one whose birth was experimentally delayed one week. (From Snyder, 1934.)

birth is proportional to the size of the dam in each case (see Fig. 21. 13, Chapter 21). The crossbred foal born of the large mother is three times the weight of the crossbred foal born of the small mother. It seems possible that the limiting amounts of some special growth substance in the blood limits the size of the foal to such as the mother can bear. Size differences between these foals are continued throughout life. Castle (1936a, b) who found evidence of such maternal influences on size in reciprocal crosses in rabbits and mice, attributes these to cytoplasmic influence of the egg. Robertson and Ray (1919, 1920) found in mice that if tethelin or cholesterol (which increases the cellular elements to the disadvantage, in the competition for nourishment, of

the connective tissue) was given only so long as they were immature, the cessation of the stimulus was followed by great increase in size. Such increase was due to

the growth of sclerous tissue, and, added to the already highly developed cellular tissue (hitherto not noticeable), resulted in gigantic individuals (*see also* Robertson, 1923).

Severe limitations of energy-nutrition of the mother, too, affect the weight of the new-born young (Hammond, 1932 ; Thomson and Fraser, 1939). This is marked in twin-bearing ewes in which if they are fed so as to lose weight during the second half of pregnancy the lambs are some 40 per cent. lighter than normal at birth (Verges, 1939). With ewes bearing single lambs this reduction of birth weight is but slight, for as with calves (Eckles, 1919) it seems probable that the mother can supply sufficient nourishment from her own body to support a single foetus.

Wallace (1948) has shown that in sheep it is the state of nutrition of the mother during the last six weeks or so of pregnancy that is the important factor in determining the size of the young at birth. This is the time when normally the foetus

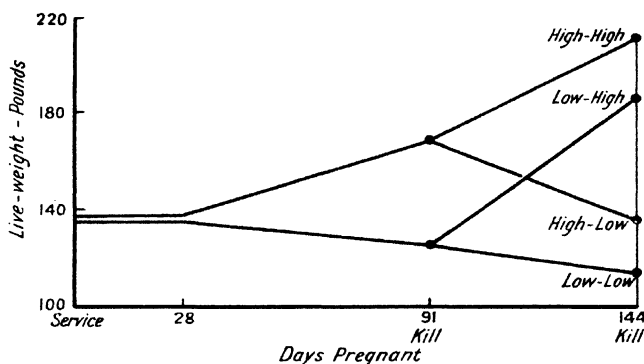


Fig. 23. 11—Live weight growth curves of ewes during pregnancy. Live weight was controlled by changes in the plane of nutrition. For results of this on the embryo see Fig. 23. 12. (From Wallace, 1948.)

itself is making rapid gain in weight. By the individual feeding of ewes on a good ration so as to make them gain or lose weight according to a predetermined plan (Fig. 23. 11) during the various stages of pregnancy and by slaughter and weighing of the foetuses at mid-pregnancy and at about 4 days before birth was due, he showed that the state of nutrition of the ewe up to mid-pregnancy had little or no effect on foetal weight, but that the rate of gain of the mother during the last eight weeks was the important factor in determining birth weight of the young (Fig. 23. 12).

In order to explain how the various factors may affect the birth weight a theory of the partition of circulating nutrients between the different tissues of the body has been put forward by Hammond (1944) and is illustrated by Fig. 23. 13. It is suggested that the partition of incoming nutrients between the different tissues and parts of the body is determined by the local metabolism rates (denoted by the number of arrows) and so by this means priorities of supply on incoming nutrients are determined in times of short supply. When, for example, incoming nutrition is slightly reduced, one arrow is removed for each tissue so that all

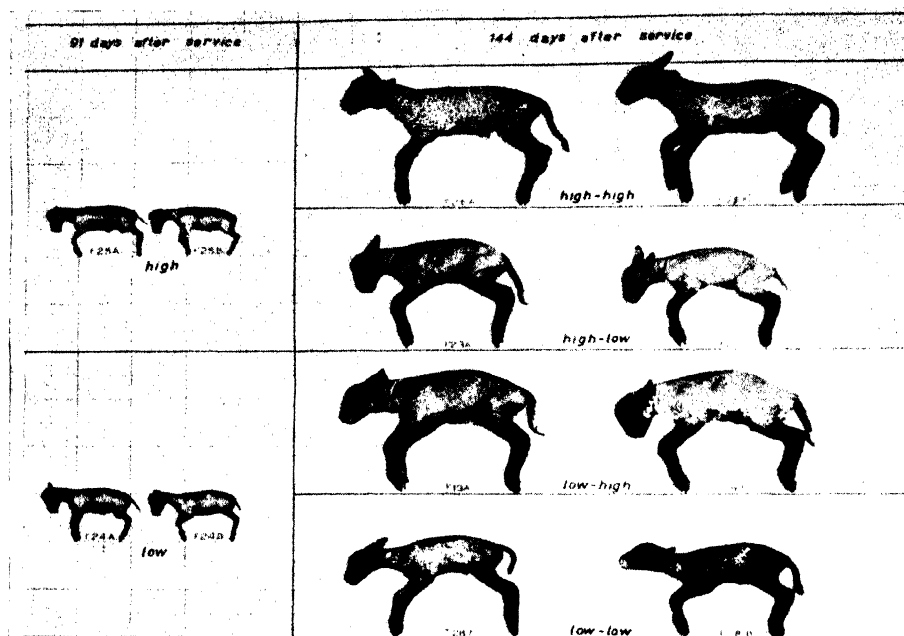


Fig. 23. 12—Effect of level of nutrition of the ewe during pregnancy (see Fig. 23. 11) upon the growth of twin embryos. There is no difference at 91 days of pregnancy, but large differences due to the plane of nutrition are shown at the 144th day. (From Wallace, 1948.)

tissues except fat continue to grow. When, however, on a lower plane of nutrition still (two arrows removed), while brain, placenta and bone continue to grow,

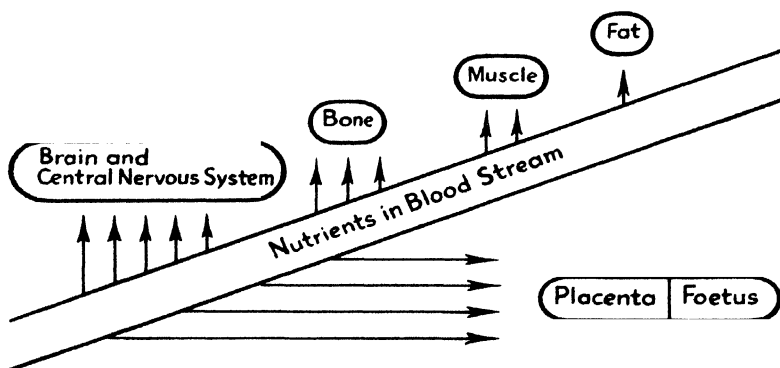


Fig. 23. 13—Priority of partition of nutrients according to metabolic rate. For explanation see text. (From Hammond, 1944.)

muscle growth ceases, and fat (arrow reversed) is removed to build up tissues with greater priority. In such a scheme it would appear that the foetus has a high priority during the early stages of pregnancy, but that as pregnancy proceeds

its relative priority to other tissues becomes reduced. As in different species of animals birth occurs at different stages of foetal development such a theory would explain species differences in the effects of nutrition on birth weight: for example, in the large effects in the sheep and horse which are born at an advanced stage of development and the smaller effect on the rat and mouse which are born in an earlier stage of development.

The practical importance of birth weight in farm animals is that weight is an indication of anatomical and physiological, as distinct from chronological, age. For example, the High-High lambs shown in Fig. 23. 12 are anatomically and physiologically older than their actual age, while the Low-Low lambs are anatomically and physiologically younger, that is, the proportions and development of the different parts and organs of the body have been advanced and retarded respectively.

Some other characteristics, such as heat regulation (Brody, 1945), are similarly affected and thus have a considerable effect on their powers of

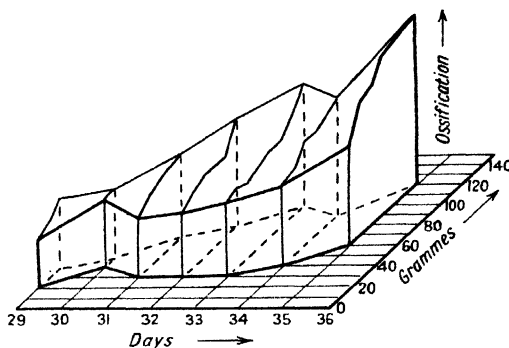
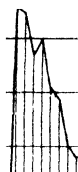


Fig. 23. 14—The relation between the stage of ossification in the limbs of the new-born rabbit and its weight and conceptional age shown as a three dimensional chart. The effect of weight on stage of ossification is greater than that of conceptional age. (From Appleton, 1929.)



Guinea Pigs
Percentage increments. Males

2 58 11 17 23 29 35 38 45 60 75 90 105 120 135 150 165 180 195 210 241
Days

Fig. 23. 15—Changes in the postnatal growth rate of the guinea-pig. This species is born at an advanced stage of development and is only capable of adding just over 5 per cent. to its weight in a single day shortly after birth, while by the 90th day the rate drops to less than 1 per cent. Compare with similar changes for the rabbit shown in Fig. 23. 16. (From Minot, 1907.)

survival after birth. Appleton (1929) has shown that in litters of new-born rabbits from an inbred strain the stage of ossification reached by the bones depends a great deal more on the actual weight of the young than on the chronological age (Fig. 23. 14.)

Thus, as Brody (1945) points out, the rate of flow of physiologic and anatomic time may be accelerated or retarded within certain limits. In the lower organisms the flow of physiologic time may be practically stopped by desiccation, especially at low temperatures (*see* Chapter 21, p. 698).

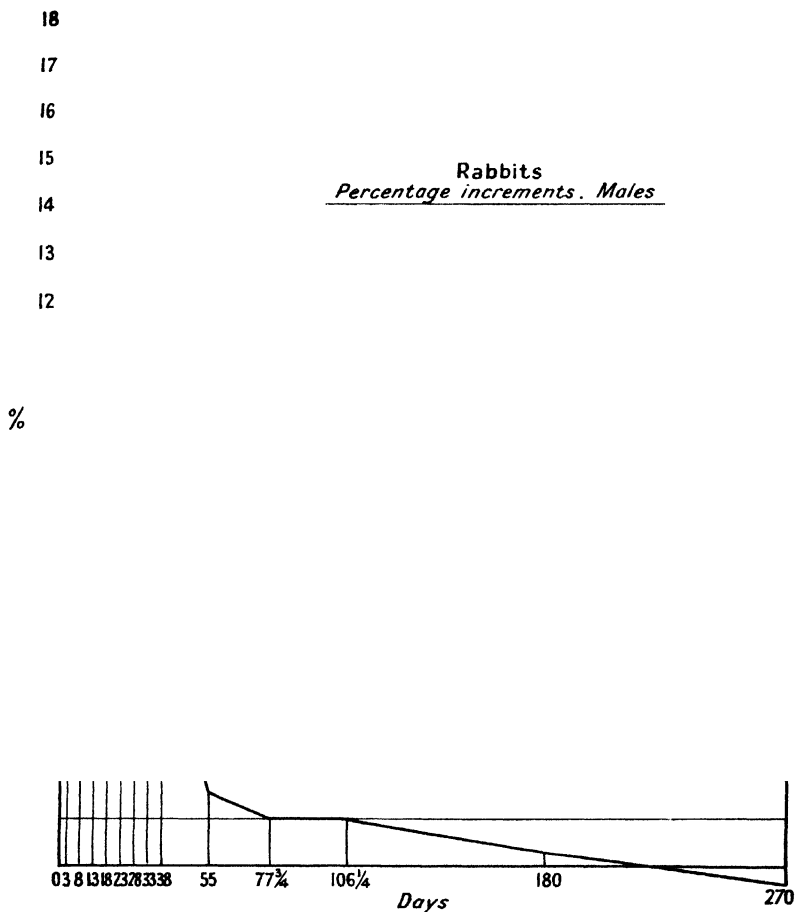


Fig. 23. 16—Changes in the postnatal growth rate of the rabbit. This species is born at a comparatively immature stage of development and adds just over 17 per cent. to its weight in a single day shortly after birth. The rate of growth falls rapidly, however, and reaches the 1 per cent. level at about 78 days. Compare with similar changes for the guinea-pig shown in Fig. 23. 15. (From Minot, 1907.)

Post-natal Growth

Minot (1891, 1907) was one of the first fully to investigate the rate of growth from birth to maturity in the guinea-pig (Fig. 23. 15). This species is born at an advanced stage of development as compared with others such as the rabbit, rat, or ferret, the period of gestation being unusually long. Immediately after birth

there is a lessening of the power of growth, which he ascribes to the physiological shock from which the organism suffers as a consequence of being born. Another factor, however, is the loss of water by evaporation which occurs shortly after birth; Richter and Brauer (1914) find that owing to this cause new-born calves, lambs and pigs remain about the same weight for two days. In man after the first few days there is, on the average, a 9.2 per cent. loss of body weight: this Feldman (1920) ascribes not only to the loss of water, but also to excretion which is not balanced by food intake, for in man the mother's milk, especially in primiparae, is often not completely established at first. By two or three days after birth Minot found that the young guinea-pigs are fully recovered and are capable of adding over 5 per cent. to their weight in a single day. By the time they are 17 days old, however, they are only able to add 4 per cent. to their weight, and by the time they are 24 days old, less than 3 per cent. When they have been born 45 days they can add only a little over 1 per cent. to their weight; when 90 days old, less than 1 per cent., and still less as they grow older, until when about a year old they attain their full size (Fig. 23. 15). Minot also investigated the rate of growth in the rabbit and in the chicken. The young rabbit is born in a very immature state of development after a relatively short gestation period. Correlated with this fact, he found that the male rabbit four days after birth is capable of adding over 17 per cent. to its weight in a single day (Fig. 23. 16); from that time the percentage increment drops very rapidly, so that at the age of 23 days the rabbit can add only a little over 6 per cent. After about the 55th day the decline in the growth-rate, which has hitherto been rapid, becomes more gradual. Similar studies on the growth of different species have been made by many workers, and reference to these will be found in the publications of Brody and his co-workers (1926a, b, c, d, 1927, 1945).

In man the type of growth curve differs somewhat from most other species (Fig. 23. 17), especially with regard to the prolongation of the juvenile period (Brody, 1927d) and to the sex differences (Key, 1890; Havelock Ellis, 1914; Dufestel, 1920; Murray, 1921). Growth is most rapid during the first year of life, when a child is able to increase its weight by 200 per cent. For the second year this percentage drops to 20 and for subsequent years up to about the age of 13 it fluctuates around 10, showing a gradual tendency to decrease. After this there is a distinct increase in the percentage increment representing the prepubertal and pubertal growth. There is then a further decline in the power of growth which gradually diminishes. In both sexes the weight of the body tends to increase until about the 50th year or somewhat later, owing to an accumulation of fat, but there are, of course, very many exceptions (Minot, 1907). Sex differences are marked in the growth curve of man. The prepubertal growth of girls usually precedes that of boys, so that between the ages of 12 and 15 girls are often heavier and taller than boys. Boys grow most rapidly at 16, girls at 13 or 14 (Fig. 23. 18). Boys attain their full height at from 23 to 25 years of age: girls at 20 or 21. These sex differences vary slightly in the different races of mankind owing to variations in the age of sexual maturity, etc., for example, the girls in Ceylon are taller than the boys for a greater length of time than British girls, and their growth slows down at an earlier age as they reach womanhood (Nicholls, 1936).

Most of the work in the field of growth studies in recent years has been on the determination of the factors which affect the normal growth curve as described

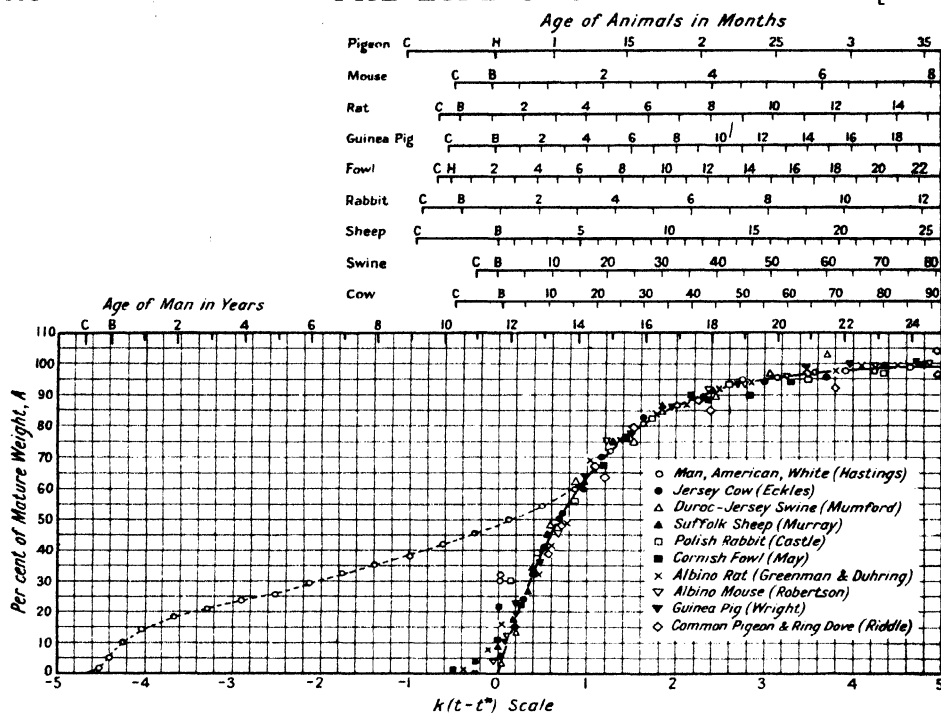


Fig. 23. 17—Growth equivalence (during the phase of growth following puberty) between man and animals. The differences (as they relate to the juvenile and infantile periods) between the curves of man and animals are infinitely greater than the difference between the curves of widely separated species of animals. (From Brody, 1927d.)

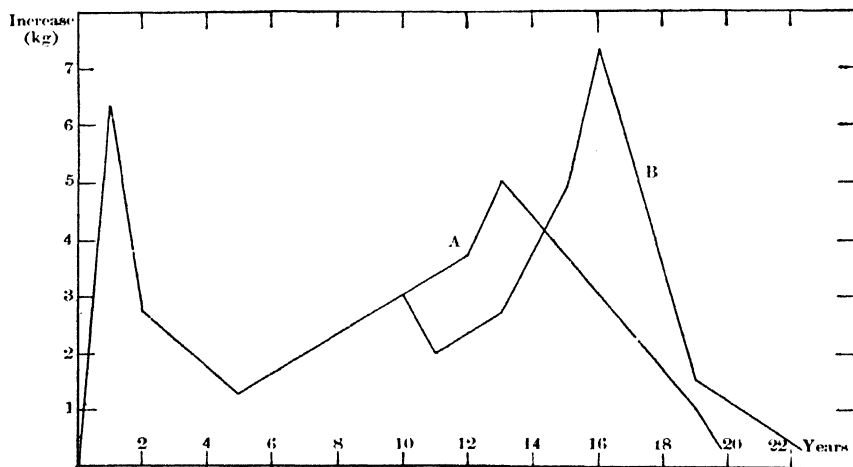


Fig. 23. 18—Growth rate (increase in kg. per annum) of girls (A) and boys (B). Prior to the tenth year the rate of growth of the two sexes is represented by the same line. Unlike most species there is in man an accelerated rate of growth at puberty, which occurs earlier in girls than in boys. (From Murray, 1921.)

above ; how it can be speeded up or retarded. Of these the nutritional level is probably the most important : this applies not only to total energy and protein nutrition but also to special accessory food substances such as certain minerals

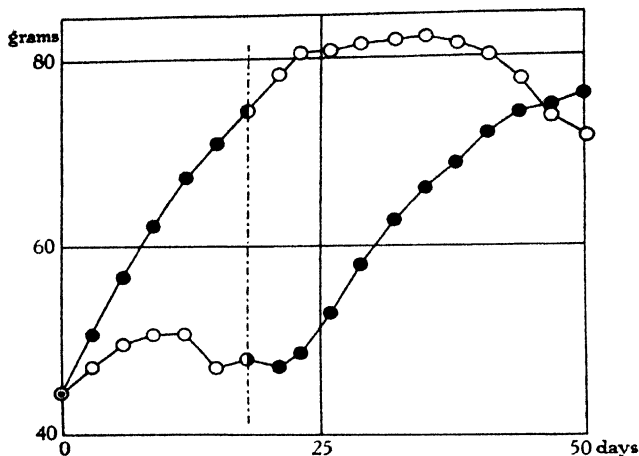


Fig. 23. 19—Growth curves of rats, with and without vitamins. ○. Artificial diet alone. ●. Artificial diet plus milk. (From Harris, 1938, after Hopkins.)

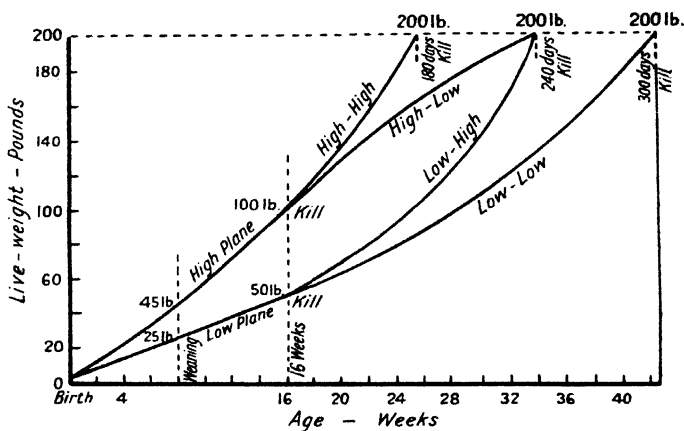


Fig. 23. 20—Growth curves of different shapes showing how the growth curves of the pig can be modified by control of the plane of nutrition (quantity of food intake) at different stages of life. For the effects of these on the conformation and composition of the pig at 200 lb. live weight see Figs. 23. 30 and 31. (From McMeekan, 1940.)

and vitamins, the addition of which to the ration in small amounts will cause a resumption of the normal growth curve in animals which have been losing weight through the absence of these factors in the diet (for literature see Sherman and Smith, 1931 ; Harris, 1938). Fig. 23. 19, for example, shows the effect on growth in the rat brought about by deficiency of the vitamins contained in milk.

In the domestic animals the shapes of the growth curves can be controlled at will, within the species limits, by the feeding of different amounts of an adequate diet. Fig. 23. 20, for example, shows the way in which McMeekan (1940) was able to control the growth curves of pigs by feeding different amounts of the same ration. Such a nutritional control of the growth curve in a species leads one to question how far the growth cycles described in some species, such for example as that described in cattle at about the age of 16 or 17 months (Murray, 1921), have any real significance, apart from seasonal changes in nutritive conditions. Variations in the milk supply are probably the most important factor in affecting the growth curve of the young animal. The effects of different quantities of milk



Fig. 23. 21—The effect of different amounts of milk intake on the size of rabbits at 28 days old. Differences in milk supply were controlled by reducing the number in the litter being suckled to one (lower), three (right upper) and five (left upper) respectively. (From Hammond, 1940.)

from the mother are most marked in the growth of lambs (*see* Fig. 23. 5). Young rabbits of the same inbred line may be grown to more than twice the normal size at weaning time (28 days) by reducing the number of young being suckled and so increasing the milk supply to the young (*see* Fig. 23. 21).

In a series of experiments in which the animals were individually fed, Wallace (1948) showed that by feeding ewes during the last six weeks of pregnancy on a high plane of nutrition the development of the mammary gland was increased as compared with those on low plane nutrition and that the subsequent milk yield (Fig. 23. 22) was greatly increased, while the rate of growth of the lambs was directly correlated with the total amount of milk produced by the groups (Fig. 23. 23), the amounts of other food being kept constant in both groups.

Aron (1913) has pointed out how, in different species, the rate of growth is proportional to the percentage of protein in the milk, and Proscher (1898) and

Belle (1930) have shown that the more rapid the growth of the young of a species the higher is the percentage of protein and ash in the milk of that species, but there is no connection with the fat or sugar content. Bunge (1887) has pointed out how the proportions of the salts (except iron) in milk are similar to those of the young animal, and that the slower the growth of the species the greater is the divergence between the two. In man, too, experiments in the feeding of extra milk to school children have shown how effective the skim milk is in causing increases in both weight and height (Orr *et al.*, 1928). Nicholls (1936) also has shown that the children of the upper class in Ceylon are, up to the age of 11 years, much the same size as the meat-eating and milk-drinking Masai tribe of Africa; whereas the lower class children of Ceylon,

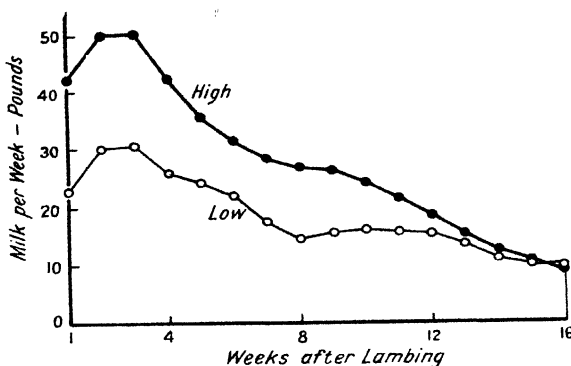


Fig. 23. 22—Milk production curves of ewes which had been fed so as to increase in weight during the last 6 weeks of pregnancy (above) and of those which had been allowed to lose weight during this period (below). For effect of this on the growth of the lambs see Fig. 23. 23. (From Wallace, 1948.)

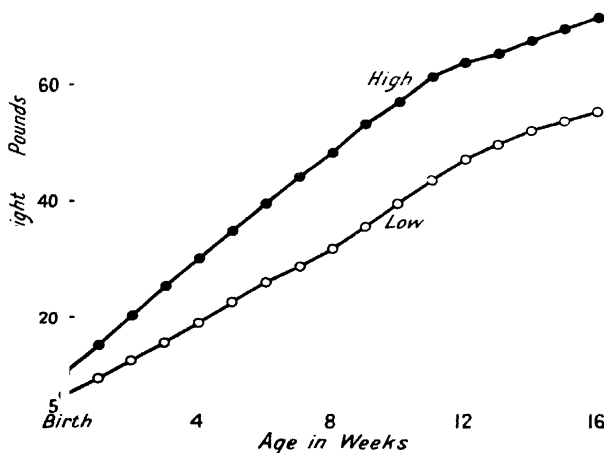


Fig. 23. 23—Growth curves of lambs from ewes giving the different amounts of milk shown in Fig. 23. 22. Food eaten, other than milk, was kept constant in both groups. (From Wallace, 1948.)

whose food is markedly inferior in quality, especially in respect of animal proteins and fresh vegetables, are of only about the same size as the children of the more or less vegetarian Kikuyu tribe in Africa.

Various opinions have been expressed as to how far a lowered plane of nutrition during growth has an effect on ultimate body size (Morgulis, 1923; Jackson, 1925) but it seems probable that it depends both on the time and duration (McCay *et al.*, 1935, 1939; Jackson, 1937) of the treatment.

The various other external factors that influence growth in animals of different kinds are referred to by Morgan (1907) and Hammond (1932) to whose writings the reader is referred for an account of the literature of the subject. In children there is a seasonal variation in the rate of gain in weight, the maximum occurring during winter and the minimum in summer (Palmer, 1933).

In the majority of mammals and birds the male is larger and heavier than the female in adult life, the guinea-pig (Minot 1907 ; Bessesen and Carlson, 1923) and the rabbit (Punnett and Bailey, 1918) being among the few species which appear to be an exception to this rule. This sex difference in size appears to fall

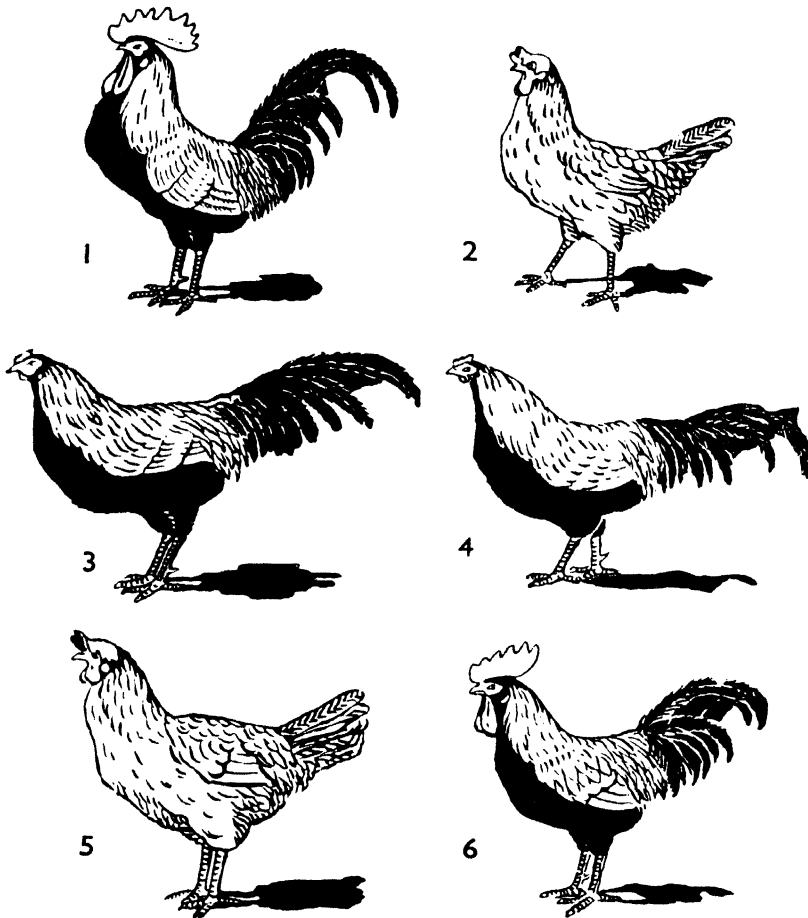


Fig. 23. 24—An experiment showing that sex differences in body size are not dependent on the gonads in Brown Leghorn fowls. 1. Normal cock. 2. Normal hen. 3. Castrated cock. 4. Castrated hen. 5. Castrated cock with ovaries transplanted. 6. Castrated hen with testes transplanted. All reduced to the same scale. (From Zawadowsky, 1931.)

into the class of sexual characters which Ellis (1914) describes as tertiary, that is, those which appear to be caused indirectly by differences in metabolism during growth rather than by direct stimulus, for such characters begin to make their appearance before puberty, they increase with age, and are not affected by castration. As an example the size (Fig. 23.24) of the castrated cock is larger than that of the castrated hen, and transplantation of the gonads of the opposite sex into these has no effect on their size (Zawadowsky, 1931 ; Finlay, 1925).

Internal control of growth in the animal is also exerted by the glands of internal secretion. The most effective of these is the growth hormone of the anterior pituitary gland. Tumours in this gland have long been known to cause gigantism and acromegaly. Evans and his co-workers (1931) among others have been able to obtain effective extracts which, when injected, will greatly increase the size of animals into which they are injected (Fig. 23, 25). After hypophysectomy longitudinal bone growth ceases and the epiphyses become closed: treatment with growth hormone prevents this (Freud, Levie and Kroon, 1939). Genetic mutations may occur which reduce the growth hormone activity of the pituitary gland

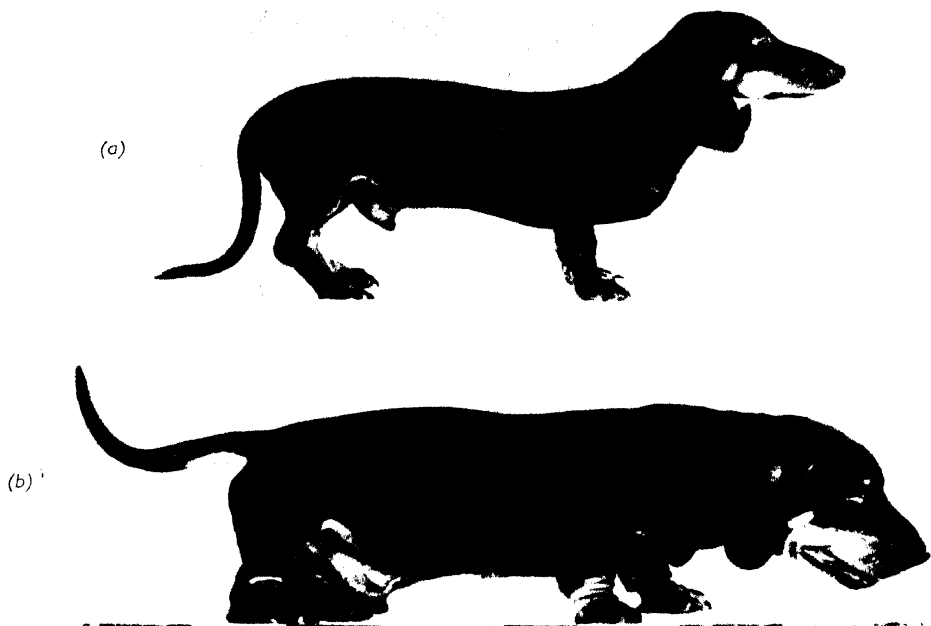


Fig. 23, 25—Changes wrought in the Dachshund by administration of the anterior hypophyseal growth hormone. The animals depicted are a pair of litter-mate brothers, untreated (a), treated (b), the latter of which had been given daily intraperitoneal injections of 10 c.c. of a purified extract of bovine anterior hypophysis for a period of six months. (From Evans, *et al.*, 1931.)

giving rise to dwarf forms (Fig. 23, 26) and these may be made to grow to normal size by the injection of anterior pituitary growth hormones (Smith and MacDowell, 1930). Whether this growth hormone is a special fraction or is a mixture of the other fractions of the anterior pituitary hormones is discussed in Chapter 12. It has been observed that during lactation cancer growths in mice are inhibited: it is possible that interrelations between the growth and lactation hormone of the anterior pituitary may be responsible for this—just as occurs between growth and sexual functions at puberty. The thymus gland, one of the few parts of the body which is larger in the infantile animal than in the adult (*see* Donaldson, 1915), has for long been associated with the growth phase of the animal, but until recently

there was little experimental proof of any effect by injection. Rowntree *et al.* (1938) found that by injections of thymus extract daily into rats including the periods of pregnancy and lactation over a succession of generations the rate of maturity of the young was increasingly accelerated so that by the eleventh generation the young grew large in size and became sexually mature and pregnant at

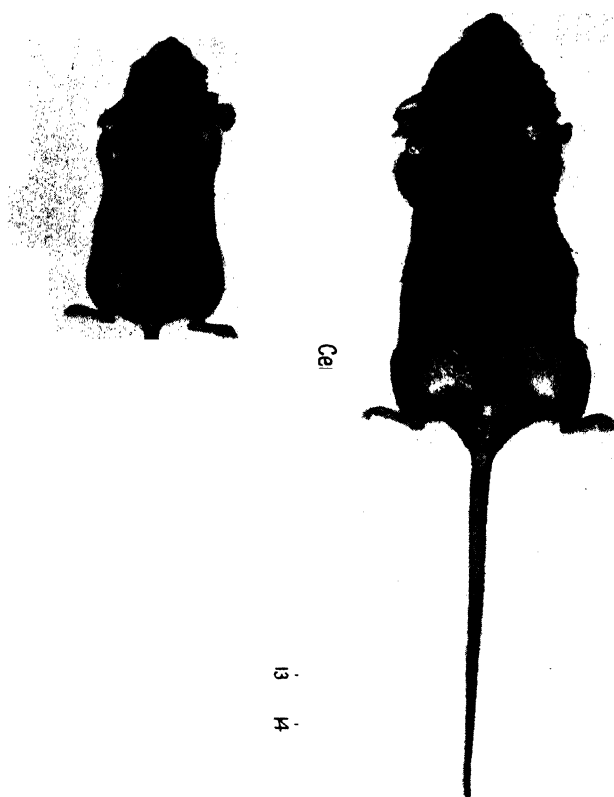


Fig. 23. 26—A dwarf form of the mouse (left) caused by a genetic mutation affecting the growth hormone of the anterior pituitary gland, and a treated (anterior pituitary gland implants) littermate (right.) (From original by Smith and MacDowell, 1930.)

38 days old as compared with 80 days in the control generation. So far their results have not been confirmed by other workers. The thyroid also has some action on the growth and mental development of the animal; deficiencies lead to cretinism, the symptoms of which can be overcome by injections of thyroxin, its active principle (Krizenecky, 1932). Simpson (1913) by removing the thyroid in lambs produced animals which failed to grow like their controls (Fig. 23. 27); similar results have also been obtained in cattle (Brody and Frankenbach, 1942; Brody, 1945). In plants duplication of the chromosomes may give rise to giant forms (Babcock and Clausen, 1927) but so far no means of doing this has been

found in animals, although as mentioned previously a halving of the chromosome material leads to the production of dwarf forms (Driesch, 1905).

During growth marked changes in the chemical composition of the body occur. The early embryonic stages are remarkable for their very high water content which gradually becomes reduced as the animal grows up—so much so, that it has been remarked that the course of life is a process of desiccation. All species of animals show a rapid decrease in water content from the time of conception until chemical maturity, which is reached at varying ages after birth (Moulton,



Fig. 23. 27—Inhibition of growth following removal of the thyroid gland in lambs. Operated (left) and normal (right) sheep. (From Simpson, 1913.)

1923). These chemical changes can be used in working out comparable ages (Brody, 1945) in different species of animals (Fig. 23. 17). While much of the decrease in water content with age is due to increase of fat, the changes mentioned above go on independently of the increase of fat with age ; the latter merely adds to the change especially in the later stages of life. For a detailed account of the chemical changes during growth, particularly in the chick, the reader is referred to Needham's (1931) work.

Developmental Growth

As the animal grows up it not only increases in size, but a definite and orderly series of changes occur in its tissues and in the proportions of its parts ; anatomical, physiological and mental characters develop and these in a general way repeat during the course of their development the evolutionary history of the species. For example, the horse (Fig. 23. 28) which has developed from a small, short-legged, marsh-living animal to a long-legged one equipped for speed on higher ground, repeats these changes in body proportions during the course of foetal development (Hammond, 1935a). After birth another series of changes occurs resulting in a

deepening and thickening of the body. Under man's selection the growth of the horse is now being evolved along two main lines—that of speed following the original evolutionary lines in the Thoroughbred, which in its body form fits on to the end of the evolutionary and embryonic scale ; and that of draught purposes, in the

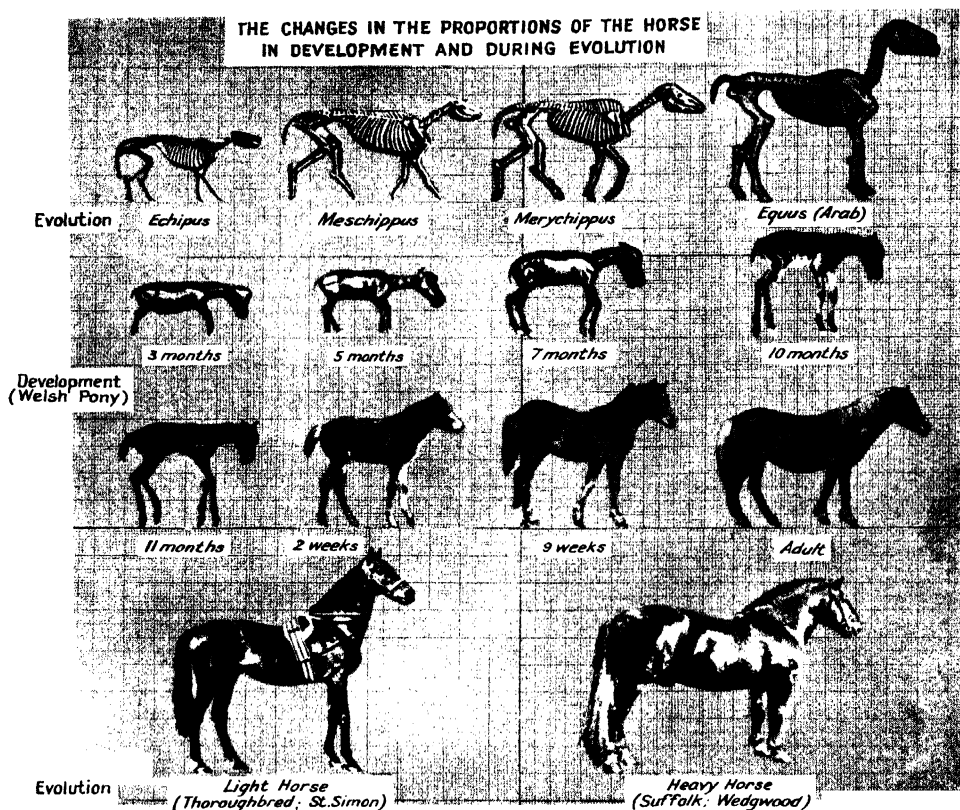


Fig. 23. 28—The changes in the proportions of the body in the horse during evolution and in the development of the individual. In order to show the changes in proportions all the photographs have been reproduced to the same cranium size (eye to ear length). The changes in proportions during embryonic life (second line) parallel those which have taken place during evolution (top line). Reading from left to right :—
Top line : Echipus ; Meschippus ; Merychippus ; Equus (Arab).
Second line : Development (Welsh pony)—3 months ; 5 months ; 7 months ; 10 months.
Third line : Development (Welsh pony)—11 months ; 2 weeks old ; 9 weeks old ; adult.
Bottom line : Evolution—Thoroughbred (an extension of second line) ; Suffolk (an extension of third line).
 (From Hammond, 1935a.)

Suffolk, which fits on at the end of the post-natal development changes (Fig. 23. 28). The development of the physiological characters of the animal during growth has been described in Chapter 17 and, for man, by Feldman (1920). For further details concerning the development of the anatomical characters of the animal, and the proportional changes of its parts during growth, the reader is referred to publications by Huxley (1932) on relative growth and Hammond (1932) on the

sheep; references to the literature are given in these works. Stockard (1931, 1941) has investigated the genetic and endocrine bases for differences in form in dogs.

Thompson (1917, 1942), in his theory of "Transformations," has shown how the main modifications of form do not occur as isolated modifications of parts of the body or skeleton, but rather that they are general ones which are spread over a number of correlated parts and originate in some deep-seated rhythm of growth: the relative proportions or co-ordinates of the whole skeleton or body are of greater importance to comparative anatomists than are the shapes of bones considered individually, so that very dissimilar forms are capable of quite a simple mathematical (and also biological) explanation (Fig. 23. 29). Huxley (1932) in his

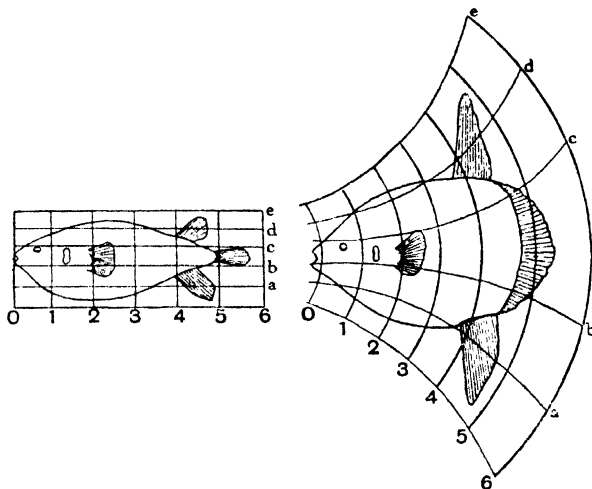


Fig. 23. 29—Cartesian transformation of the outline of the teleost fish *Disdon* (left) to give the outline of the sunfish *Orthogoriscus* (right). An illustration of how different growth gradients can alter the form of a species. (From Huxley, 1932.)

book on heterogenic growth has shown how these changes in body form as the animal grows up are capable of being described by simple mathematical formulae.

To explain such changes in the form of animals as they grow up, Child (1915) suggested a theory of growth gradients, that is, localised areas of high and low metabolic rate with gradients between them, which cause a differential growth-rate in different parts. These gradients apply not only to different areas of the body, such as running from head to rump and from distal to proximal ends of the limbs in the young animal, but also among the different tissues of the body (see Fig. 23. 13) such as gradients from brain to bone, to muscle, and then to fatty tissues (Hammond, 1932, 1944). Kestner (1934), for example, has shown that the brain, an organ which develops early in the life of the animal, has the highest metabolic rate of any tissue in the body, while fat has the least. It is found, therefore, that in general not only are the parts with the higher metabolic rate more early developing in the animal, but that they suffer less than the later developing and lower metabolic rate parts when the young growing animal is put on a low plane of

nutrition. As with growth as a whole, the plane of nutrition is a very important factor in controlling the proportions of the body and its tissues as the animal grows up. The experiments by Waters (1909), Trowbridge, Moulton and Haigh (1918) on cattle, Aron (1910, 1911, 1921) on dogs and children, and Henseler (1914) on pigs, among others, have shown how the body proportions of the animal can be changed by the plane of nutrition on which it is reared.

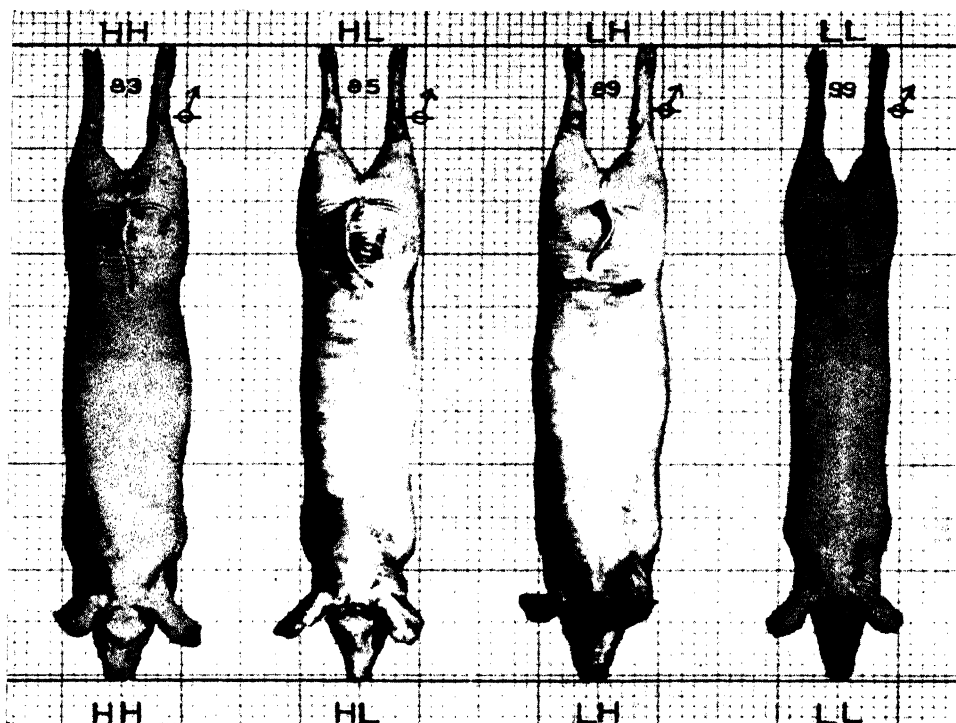


Fig. 23. 30—Experiment showing how the shape of the growth curve of an animal can change its body proportions. All the carcasses are from pigs of the same live weight (200 lb.) and are reproduced to the same body length. The growth curves producing these types are shown in Fig. 23. 20.

83 HH. Quickly grown throughout : 168 days old.

85 HL. Quickly grown to 16 weeks : afterwards slowly grown : 196 days old.

89 LH. Slowly grown to 16 weeks ; afterwards quickly grown ; 196 days old.

99 LL. Slowly grown throughout ; 315 days old.

(From McMeekan, 1940.)

In his experiments in controlling the growth curve by altering the level of nutrition in pigs at different times during the animal's life (Fig. 23. 20) McMeekan (1938, 1940) found that he could produce from the same inbred litter of pigs, at the same body weight, animals of quite different body conformation and composition. On a high plane of nutrition (Fig. 23. 30—HH) the later developing parts of the body, such as fat, were speeded up in development, whereas under a low plane of nutrition (Fig. 23. 30—LL) the earlier developing parts such as the bones grow at the expense of such later developing parts as fat.

Within any one species the growth curves of individuals tend to run parallel to one another and not to cross (Robertson, 1922) so that there is usually a high degree of correlation between the weights of the same individuals at one age and another. As between species, however, this does not hold, for although, for

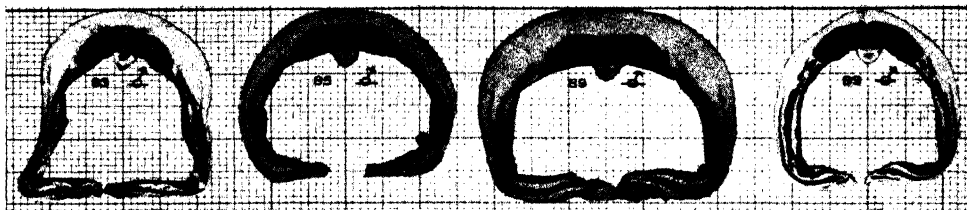


Fig. 23. 31—Photographs showing how the shape of the growth curve of an animal can affect the proportions of the tissues and composition of the body. The pig carcasses have been cut through at the last rib and are reproduced to similar longissimus dorsi muscle length. See Fig. 23. 30 for explanation. (From McMeekan, 1940.)

example, the donkey and the Large White pig have approximately the same adult weight, the shapes of their growth curves are quite different; the donkey is large at birth and grows slowly, whereas the pig is small at birth but grows quickly (Meek, 1901). It seems probable that such differences in the shape of

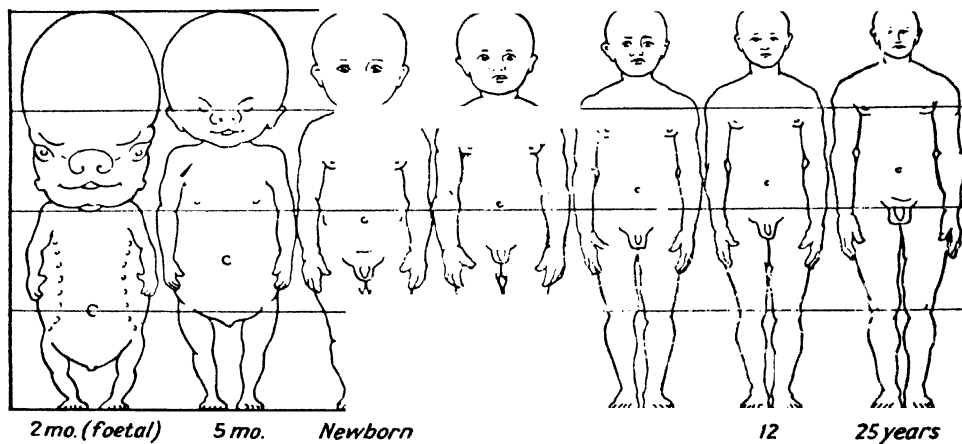


Fig. 23. 32—Changes in the form and proportions of the human body during foetal and postnatal life. (From Robbins, *et al.*, 1928.)

their growth curves are related to the differences in their adult form ; the donkey has large head and legs (early maturing parts of the body) whereas the pig has a large development of the torso (a late developing part) as compared with head and legs. Within one species this has been demonstrated experimentally by Dunlop and Hammond (1937) in the rabbit, and McMeekan (1938, 1940) in the pig. The latter, by controlling the shape of the growth curve by changing the plane of nutrition at different periods of the animal's life, was able, at the same age and body weight, to produce pigs differing very much in body proportions and

composition. For example (Fig. 23. 20), pigs on a high followed by low plane of nutrition (Fig. 23. 31—85 HL contain 20 per cent. more bone, 15 per cent. more muscle and 30 per cent. less fat than those on a low followed by a high plane of nutrition (Fig. 23. 31—89 LH).

In man the main anatomical changes in body proportions during growth are shown in Fig. 23. 32. These, too, are under the control of nutrition and it has been shown that the latter not only affects the physical but also the mental development; well-developed children are more efficient mentally and take better places at school than ill-developed and badly nourished ones (Porter, 1893). A study of twins (Newman *et al.*, 1937) has shown that late maturing characters such as weight and certain mental traits are more susceptible to environmental conditions than are early maturing characters such as stature and head measurements. The history of both civilisations and religions suggests the view that man himself, just as he has moulded his domestic animals, can guide his own evolution both mentally and physically by the environment which he himself creates.

II. PUBERTY

Puberty, or the period at which the organism becomes sexually mature, is marked by the occurrence of those constitutional changes whereby the two sexes become fully differentiated. Sex differences do occur before this time but they are of a kind called by Ellis (1914) tertiary sex characters (*see* p. 814). It is at the puberty period that the secondary sexual characteristics first become conspicuous, and the essential organs of reproduction undergo a great increase in size (Disselhorst, 1908, for man; Deanesly and Parkes, 1933, for the grey squirrel; Allanson, 1934, for the hedgehog; Brambell, 1935, for the shrew; Deanesly, 1935, for the stoat; and Brambell and Rowlands, 1936, for the bank vole). In those animals in which, during immaturity, the testicles remain within the body cavity, it is at puberty that these organs first descend into the scrotal sacs. The puberty acceleration in growth which takes place in man has already been referred to (p. 809). This change is accompanied, as is well known, by alterations in the general proportions, associated with an increase in strength, a deepening of the voice, and a growth of hair on the face and other parts of the body, processes which are not completed until about the 25th year. The breaking of the voice occurs to a certain extent also in girls but is less sudden. Barth gives the following table for the length of the vocal cords at different ages (from Feldman, 1920):—

Age in years	.	.	.	.	.	0	2	6	10	14	20	30
Males—mm.	.	.	.	.	.	6	8	10	13	13	24	30
Females—mm.	.	.	.	.	.	6	8	10	12	12	16	20

It is thus seen that the lengthening of the cords even in boys is not absolutely sudden, but extends over a number of years. In temperate climates puberty begins in boys at about the 14th or 15th year: in tropical countries it is usually a few years earlier (Steinach and Kammerer, 1920). For an account of these and other matters concerning the growth differences between man and woman the reader is referred to a book by Ellis (1914). It is at puberty that ripe spermatozoa first make their appearance in the seminal fluid which is henceforward secreted in considerable quantity.

In women puberty occurs at a slightly earlier age than in the male sex. The constitutional changes characterising this period take place more suddenly in the female, the girl almost at once becoming a woman, whereas a boy is several years before he develops into a man, complete maturity not being reached until about the 25th year. Moreover the onset of puberty in the girl is marked more precisely by the coming of menstruation, which may make its appearance in temperate climates in the 13th year. At about the same time the pelvis widens, and other characteristic anatomical changes take place; the subcutaneous layer of fat, the development of which assists so largely in giving the body its graceful contour, is deposited; while the internal generative organs enlarge and ripe ova and corpora lutea are produced by the ovary. Growing follicles, however, are by no means uncommon in the ovaries of young children. Runge (1906) states that after about the third year there is a progressive increase in the size of certain of the follicles until the ovaries become scarcely distinguishable from those of adults except for their smaller size and absence of corpora lutea. Cases of sexual precocity in girls associated with hyperplasia of the ovaries have been described by Blair Bell (1920).

In both sexes the purely physical changes of puberty are accompanied by psychical ones which are no less pronounced. Both kinds of change are dependent largely, if not entirely, upon the functional development of the generative glands (Hall, 1904).

It is a well-known fact that women marry and bear children earlier in warmer countries than in cooler regions. This has led to the assumption that girls in tropical and sub-tropical regions menstruate at a comparatively early age. Chopra (1938), from a consideration of the evidence concludes that there is a definite depression of the gonadal activity of females in the hot and humid regions of the tropics. Curjel (1920) found that the average age of the onset of puberty was 13.6 years in Indian women, while Kennedy (1933) from collected case records found the average age of onset to be 14.1 in China, 14.2 in Rio de Janeiro, 15.0 in Rome, 15.0 in Edinburgh, 15.2 in Russia, and 16.2 in Prussia. Englemann (1901) found that the American girl matures at an earlier age than the girls of any other land in the temperate zone. Yang and Gear (1934) noted that in 57 per cent. of cases reported in China the first menstruation occurred in spring and summer. Ellis (1930) states that town girls menstruate earlier than country girls, while Shaeffer (1908) finds that the age of puberty averages 14.4 years in the upper classes as compared with 16.2 years in the lower classes. Crew (1929) in summarising the general evidence at the date of writing, concludes "it would seem that throughout the world puberty is reached at more or less the same age and that the actual age at which different individuals attain it is determined by such agencies as genetic or regional modification of the glands of internal secretion, by general nutrition, and by exercise."

In animals the general nature of the change which sets in at puberty is similar to that occurring in the human species, and the secondary sexual characters often appear for the first time at this phase of life. On the whole, however, the changes occurring in animals at puberty are not so marked as they are in man. In animals, too, as in man, complete sexuality is not acquired all at once in either sex (Crew, 1931). Punnett and Bailey (1918) found that, contrarily to what occurs in man, there is a slowing down of body growth at the time of sexual maturity in the rabbit.

In only a few wild species of animals has the actual age of puberty been determined with any degree of accuracy ; some of these are shown in the table on p. 794 (from Asdell, 1946). In most wild animals the young are only born at certain definite times of the year and so in the small species which are early maturing the age of puberty is more or less fixed by the following breeding season (Deanesly and Parkes, 1933, for the grey squirrel ; Deanesly, 1934, and Allanson, 1934, for the hedgehog ; Brambell, 1935, for the shrew ; Deanesly, 1935, for the stoat ; Brambell and Rowlands, 1936, for the bank vole ; and Brambell and Hall, 1936, for the lesser shrew). In the case of domestic animals the information obtained from general practical experience is available, but there are few or no exact data. Most fillies come in use within two years and all by the time they are three. Heifers may come on heat when they are a year old, but service is generally postponed until three months or more later. Sows will receive the boar when they are six months old, and sometimes two months earlier. The white mouse is stated to breed when six weeks old (Kirkham, 1920) and the white rat at between 37 and 55 days (Evans and Bishop, 1922.)

The primary cause for the onset of the puberty changes does not rest with the age of the ovary, for as Foà (1900, 1901) has shown by reciprocal transplantation of ovaries between infantile and adult rats, the age of the body and not the age of the gonad determines whether the gonad will function or not. It seems probable that changes occurring in the anterior pituitary gland determine the time at which the onset of puberty and the functioning of the gonad occur (*see* Chapter 12), for injections of extracts of this gland will cause puberty changes to occur in the gonads (Zondek and Aschheim, 1927) when an animal, such as a mouse, is only in the infantile stage. Similarly, development of the secondary sexual characters such as the growth of the comb and crowing can be produced in a chick at only 9 days and treading at 13 days after hatching, by injections of anterior pituitary extracts (Domm, 1934). Improvement for egg production in fowls is often attended with earlier sexual maturity ; thus Hall and Marble (1930) found that after breeding Leghorns for high egg production the age at which the first egg was laid was reduced to about 200 days as compared with 240 days for their low egg production strain. In male chickens and turkeys there is some evidence for the inheritance of early sexual maturity (Lorenz and Lerner, 1946). The various factors which affect the time of the appearance of puberty probably act through the pituitary-gonad mechanism. Such exteroceptive factors, too (*see* Chapters 1 and 13), as the length of daylight changes during the course of the year which cause the onset of the breeding season will accelerate or retard the age of puberty according as the normal age for puberty in the individual falls before or after this season. Pullets, for example, if hatched too late in the spring so that their normal age of puberty falls during the early winter months, when egg production is at its lowest ebb, will not begin to lay at so early an age as those which are hatched earlier in the year (Goodale, 1918). The age, too, at which puberty is attained in rabbits as measured by the time at which first conception occurs varies regularly with the month during which they are born, being longest (30 weeks) for those born in March–April and shortest (23 weeks) for those born in October–November. The normal breeding season of the British mutton breeds of sheep extends from September to March and those lambs which are born early in the season will be longer at arriving at the age of puberty than those which are

born later in the season. It has been shown (Hammond, Jr., 1944) that in sheep there is neither a fixed age of puberty nor a fixed time of year for the first heat. The threshold of stimulation required falls in the lamb as its age increases, until at about 300 days it reaches the adult level. The intensity of stimulation increases as the amount of daylight lessens and is maximal at the middle of the breeding season; thereafter it decreases at a greater rate than that at which the threshold for the lamb falls with increasing age—so that the minimum age at first heat comes in the middle of the breeding season, the age being about 180 days: if this age is reached later in the season heat will not occur until the season following when the animal may be 400 days or more old. Such exteroceptive factors also probably account for the fact that puberty in man may be reached at an earlier age in the tropics than in temperate zones, although it should not be overlooked that in most cases the smaller breeds within a species attain the age of puberty earlier than do the larger breeds. For example, the small Polish rabbit attains sexual maturity (age at first conception) at about 24 weeks old as compared with the larger Belgian hare which does not reach puberty until it is about 30 weeks old. Similarly it is said that heifers of the small Jersey breed of cattle come into oestrus before those of large breeds such as the Welsh. Small dogs will breed at from 7 to 10 months old, but the larger kinds do not breed so soon. Small breeds of fowls such as the Leghorn produce their first egg in about 200 days whereas large breeds such as the Brahmas take about 290 days (Waters, 1934). Moreover, it has been shown in fowls that selection for body size within a breed delays the onset of sexual maturity (Lerner, 1946). These connections between body size and age of puberty may be due to interactions between the growth and gonadotrophic hormones of the anterior pituitary. In this connection the interrelations between nutrition, growth and egg production in fowls are interesting. Prentice, Baskett and Robertson (1930) found that by good nutrition of the growing pullet the age at which the first egg was laid (135 days) was considerably reduced as compared with poorly nourished birds (186 days), but that the poorly nourished ones, if hatched at the right time of the year, began to lay at lower body weights than the well-nourished birds. Studies of such interactions between growth and the age at which reproduction begins are of importance in view of the fact that among the Primates the lengthening of the childhood period and delaying of the age of puberty (Fig. 23. 17) has been one of the factors associated with the evolution of the higher forms of mental development. The brain is an early maturing part of the body (*see* p. 819) and it seems probable that the long infantile period gives a better chance for its full development.

III. THE MENOPAUSE

In the male sex there is no definite age at which the reproductive functions cease. In the female on the other hand the close of the reproductive period is far more definite, and it is this change in a human female which constitutes the menopause or climacteric. The essential phenomenon of the menopause, therefore, is the permanent arrest of all the functions connected with reproduction, although cases are on record of women conceiving some years after the apparent menopause. It is the inversion of the developmental process of puberty and marks the termination of actual sexual life. In temperate climates it almost always takes

place between the ages of 40 and 50, and most usually at about the age of 45 (for further details *see* Kelly, 1908, and Luciani, 1921). In warm countries it has a tendency to be earlier and in colder ones later. It is usually earlier among the labouring classes and also in women in whom puberty was early. The actual duration of the period when menopause symptoms occur varies from about three to five years.

The symptoms of the menopause may be referred to two stages—(1) a stage of menstrual irregularity, and (2) a post-cessation stage, during which various systemic disturbances are wont to occur. During the latter period especially,

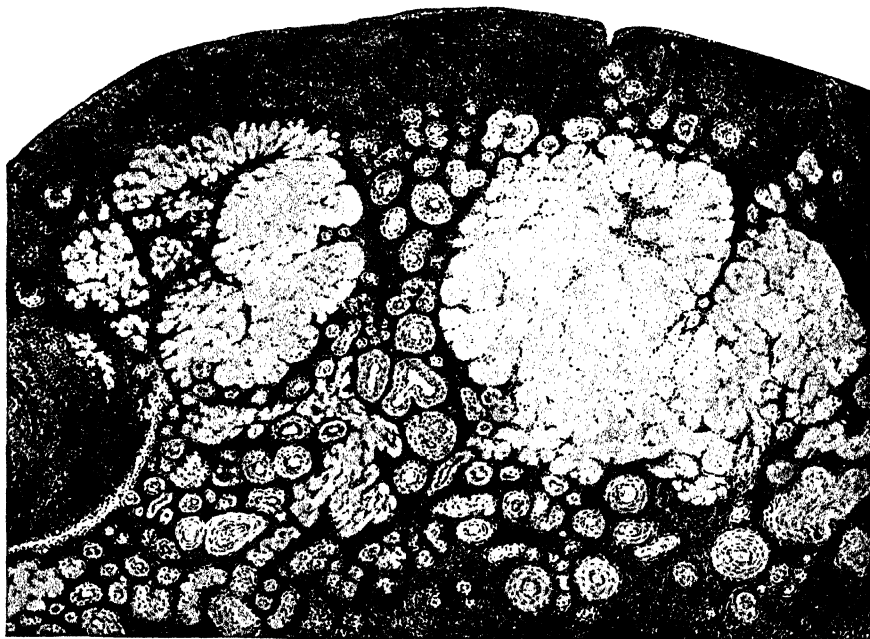


Fig. 23. 33—Section through ovary of woman of fifty-six, showing degeneration of follicles and sclerosis of connective tissues. (From Sellheim, 1907.)

the organic functions are irregular. Palpitation, dyspepsia, sweating, and vasomotor changes are not infrequent, and neurasthenic phenomena, hysteria and other psychic disturbances sometimes occur, accompanied by neuralgia, rheumatism and various disorders. Mental instability at the “change of life” is not uncommon. It is well known that the menopause is followed by profound psychological changes which vary in different individuals.

The atrophy of the reproductive organs (Dudley, 1905; Sellheim, 1907) is the main anatomical and physiological basis of the menopause. Senile changes take place in the ovary, consisting of atrophy, induration and shrinkage to the size of the rudimentary ovary, the disappearance of Graafian follicles and the cessation of ovarian function (Fig. 23. 33). In the fallopian tubes the senile changes comprise a destruction of the epithelial cells, together with a shortening and a narrowing of the tube, often accompanied by obliteration of the lumen. The senile changes

in the uterus consist of atrophy of the entire organ, which may be reduced to a hard wedge-shaped body one-quarter of the size of the functional organ ; in many cases closure of the external or internal os or complete obliteration of the canal,



Fig. 23. 34—Section through uterine mucous membrane of woman of sixty. *gl*, Glands.
(From Sellheim, 1907.)

consequent secretions producing pyometra or hydrometra due to locking up of the secretions ; in some cases the disappearance of the vaginal portion of the cervix, making the upper part of the vagina continuous with the uterine canal ; degenera-

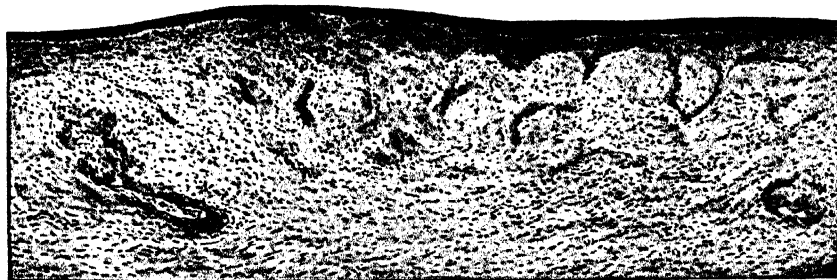


Fig. 23. 35—Section through vaginal mucous membrane of woman of sixty-one.
(From Sellheim, 1907.)

tion of the muscular and glandular elements (Fig. 23. 34) ; and cessation of menstruation. In the vagina senile changes consist of a shortening and a narrowing of the organ with loss of elasticity ; the stratified squamous epithelium is lost and is replaced by a hard surface containing cicatrical tissue (see Fig. 23. 35) ; and contraction of the entrance to the vagina. The senile changes in the vulva comprise

great contraction and loss of elasticity and destruction of glands and follicles, while the cutaneous surface becomes dry and scaly. In the mammary glands the senile changes consist in a loss of the glandular elements and cessation of function, together with shrinkage due to atrophic loss, which, however, is sometimes compensated for by a deposition of fat.

Other changes, depending possibly, but by no means certainly, on the degeneration of the ovaries, are the assumption of certain of the secondary male characters such as the growth of a beard especially above the corners of the mouth (Lipschütz, 1924), and changes brought about in the other organs of internal secretion (Blair Bell, 1920). At the menopause changes also occur in the urine and a hormone appears therein which has active gonadotrophic properties. This would seem to suggest that the cause of the menopause originates in the inactivity of the ovary itself rather than in the anterior pituitary mechanism which other-

wise controls the ovaries (see Chapter 12 for further details). At the menopause there is sometimes a distinct increase of sexual desire.

No definite ages at which the various species of animals cease to breed can be given as most die before reaching their climacteric. Out of 1,216 Thoroughbred mares recorded in the earlier volumes of the *General Stud-book*, one bred at 33, two at 30, four at 29, seven at 28 and seventeen at 27; Wood (1921) concludes that the mare generally continues to breed as long as she lives.



Fig. 23, 36—A pedigree Guernsey cow (Lady Shamrock) 21 years old with her calf. (From *Amer. Guernsey Breeders' J.*, 1 Jan., 1945.)

Sheep have been known to produce young up to 20 years and cats to 14, but they may cease somewhat earlier and yet maintain a healthy existence for a few years (Fleming, 1912). Kirkham (1920) says the white mouse stops reproducing at 18 to 22 months after having 12 to 16 litters. The menopause in the white rat occurs at the age of 15 to 18 months (Donaldson, 1915). In the rabbit the ovulating mechanism seems to disappear before the power of ripening follicles: this also frequently happens in aged mares. For most other species there has been little systematic research and the only evidence of the age to which various species will breed is to be found in letters to the daily papers or periodicals reporting what appears to be an exceptional case. For example, a ferret at seven years old produced a litter of six (*Country Life*, 83, 30 Apr., 460); a cock pigeon at 17 years old was breeding excellent youngsters (*Racing Pigeon*, 1944, 7 Oct., 105). With cows there are many records of pedigree animals which have produced calves at from 15 up to 21 years old (*J. Red Poll Breed*, 1943, 20 Dec., 40; *Amer. Guernsey Breeders' J.*, 1944, May, 769, Sept., 381; 1945, Jan., 9, Aug., 289, Nov., 1,009); one of these, with her calf, at 21 years old is shown in Fig. 23. 36. Isolated cases of

cows breeding at even older ages are also reported ; thus Housman (1897) refers to the Aberdeen Angus cow " Old Grannie " owned by Hugh Watson of Keillor : she lived to be 35½ years old and had 25 calves.

IV. SENESCENCE

As age advances, in addition to the menopause changes which relate more especially to the cessation of the female generative functions, atrophic changes of one sort or another take place in both sexes throughout practically the entire system.

As has previously been pointed out (p. 819) the various organs, parts and tissues of the body develop in a definite order and, according to Child (1915), have different metabolic rates. The earlier developing and higher metabolic-rate parts therefore suffer less from a low plane of nutrition than do the later developing and lower metabolic-rate parts. Thus in the downward curve of life we might expect those organs and parts to go into senility in the reverse order from that in which they came. The best description of this for man has been given by Shakespeare :

" The sixth age shifts
Into the leane and slipper'd Pantalooene,
With spectacles on noze, and pouch on side,
His youthful hose well sau'd, a world too wide,
For his shrunk shanke, and his bigge manly voice,
Turning again towards childish treble pipes,
And whistles in his sound. Last scene of all,
That ends this strange eventful historie,
Is second childishnesse, and meere oblivion,
Sans teeth, sans eyes, sans taste, sans everything."

As Gilford (1911) has pointed out, senility in man is a regression to a second childhood both physically and mentally, apart naturally from weathering, and tissues which are rigid and so cannot be absorbed. The decline of development in the nutrition of the tissues with age causes growth to become unbalanced, or irregular as age advances ; for example, there is an increased frequency of cancer growth with age. In the male sex the prostate gland undergoes atrophy, or in some cases a pathological hypertrophy, which is said to be the cause of frequent penile erections; the enlarged prostate prevents the bladder from being properly emptied.

The rise of growth in early life and the equilibrium of maturity are followed by the leanness or fatness of middle age, this being still more accentuated in old age.

The sex organs, the last to develop in life, are the first to go, while the kidney and heart, which develop early in life, retain their size in old age (Ribbert, 1908). The heart in fact is usually slightly enlarged in old age ; this apparent hypertrophy, however, is not associated with an accession of powers, but, as with muscular tissues in general, with an increased feebleness, and the pulse, in order to compensate for this, as in the infant, beats more quickly, the normal rate of 72 beats per minute rising to 79 or 80. In old age the adipose tissue beneath the skin disappears, especially in advanced old age, but fatty degeneration of muscles or glandular tissue is not infrequent. The muscles become weaker and they shrink

in volume, the fibres of which they are composed becoming smaller and smaller in size and fewer in number. The teeth decay and drop out. The pigment of the hair undergoes absorption, the hair turning grey or white and often falling out. The rate of respiration rises slightly and the vital capacity of the lungs diminishes, while the amount of carbon dioxide excreted becomes less. The size of the liver and other viscera undergoes diminution. The internal spongy structure of the bones is dissolved away, so that they are left with only a hard external shell and consequently become brittle. The arterial walls lose their elasticity and undergo sclerosis, a characteristic which is so constant that it has given rise to the well-known dictum that "a man is as old as his arteries." The tendons and ligaments also become calcified, and there is a constant shrinkage of stature.

It has been shown also that the brain decreases in size in old age. The shrinkage begins soon after maturity and then continues almost steadily to the very end of life (Minot, 1907). Handmann (1906) gives the following table :—

Age—years	Weight of brain—grs.	
	Man	Woman
4-6	1,215	1,194
7-14	1,376	1,229
15-49	1,372	1,249
50-84	1,332	1,196

The decrease in brain weight is accompanied by a diminution in the thickness of the cortex and in the number of tangential fibres present in it. These changes are associated on the psychical side with a gradual mental failure—loss of memory, decrease in the power of original thought and in the assimilation of new ideas, and general decline of mental activity. Moreover, the reaction time is lengthened, the sense organs lose their delicacy, and the eye power of accommodation is largely lost.

The minute cellular changes in the tissues are no less pronounced. These also are in the direction of atrophy. There is a general shrinkage in the protoplasm of the cells, but especially in the nuclei, so that the relative amount of cytoplasmic to nuclear substance becomes increased in old age. The nucleoli also tend to disappear. Hodge (1894) has made a comparison of the changes in the cells of the first cervical ganglion with the following result :—

	Volume of Nucleus	Nucleoli observable in Nucleus in
At birth	100 per cent.	53 per cent.
At 92 years	64 per cent.	5 per cent.

The nuclei, besides becoming smaller, grow irregular in shape, and in the cytoplasm there is a deposition of pigment granules (Figs. 23. 37 and 38).

Senescence in men is said to commence at about the age of 50 (Lee, 1896) but no definite limit can be assigned to the period. In women the period of senescence is usually reckoned from the menopause (Hall, 1922). Spermatozoa may continue to be produced even in quite advanced old age, and instances have been recorded of men of 94, 96 and even 103 in whose semen active sperms were found (Cooper, 1908). A case has been recorded (*The Times*, 1936, 14 Sept.) of a man who lived to be a 100 and had a son when 95 and a daughter when 97. A pedigree Guernsey bull of 21 years old still serving cows has been recorded by Becker and Arnold (1941). There can be no doubt, however, that the spermatozoa are produced in far less abundance in old age. Sanders (1926) found that the fertility of stallions, as measured by the number of conceptions to mares served, was reduced in old age. Arguing that the loss of reproductive

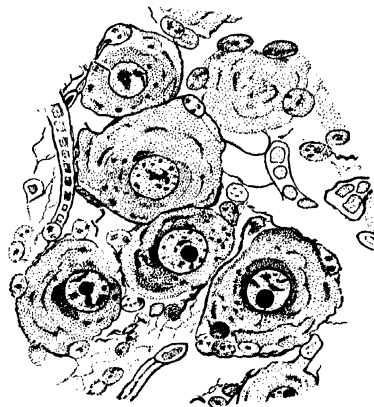


Fig. 23. 37—Group of nerve-cells from the first cervical ganglion of a child at birth. (From Minot, 1907.)

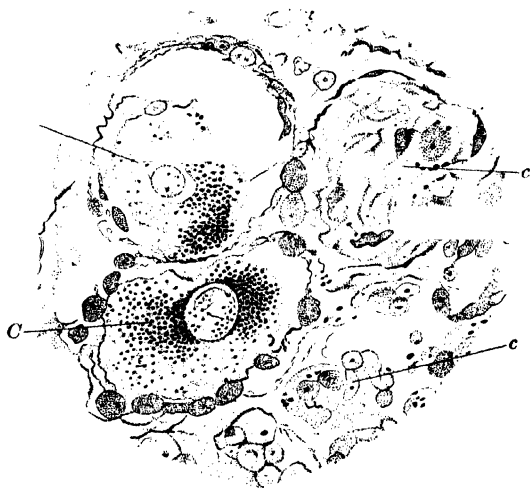


Fig. 23. 38—Group of nerve-cells from the first cervical ganglion of a man of ninety-two. C, C, cells still intact, but shrunken and loaded with pigment; c, c, cells which have been disintegrated. (From Minot, 1907.)

activity led to senescence of the body as a whole, rather than the reverse, Steinach (1920), by the operation of vasectomy in rats and other animals, and Voronoff (1923) by the transplantation of monkey or other testes into the scrotum of man or other animals have attempted to rejuvenate senile individuals by increasing their sexual activity.

It is difficult to form any accurate comparison between the later phases of life of men and those of animals, partly because so little is known regarding the condition of natural senescence and death in animals. The length of life in domestic animals is usually determined by the wearing out of some part or organ of the body which lowers their efficiency: thus in sheep as soon as the teeth go the

animal fails to thrive under poor nutritive conditions and is killed. Smith (1907) remarks that few horses live long enough to show much sign of arterial degeneration. The work they perform is the chief cause of their rapid decay, for their legs wear

out before their bodies. But apart from this, degenerative changes in the teeth, and more particularly the wearing away of the molars, prevent many horses from reaching a real old age. Blaine (1858) has drawn the following comparison between the age of a horse and that of a man : " The first 5 years of a horse may be comparatively considered as old as a man of 20 ; a horse of 10 years as a man of 40 ; a horse of 15 as a man of 60 ; of 25 as a man of 70 ; of 30 as a man of 80 ; and of 35 as a man of 90." Both Brody (1927d, 1945) and Pearl (1922) have drawn up tables to show the comparative ages in other species of animals. An account of the problems of ageing, not only in the different tissues and organs of the body, but also on the chemical and psychological problems of ageing, has been published by Cowdry (1939) : to this work the reader is referred for further details.

V. THE DURATION OF LIFE AND THE CAUSE OF DEATH

Weismann (1891), in a famous essay on the duration of life, and Metchnikoff (1907), in his book of optimistic studies, have dealt at some length, but from different standpoints, with the factors which determine longevity in the animal kingdom. That the duration of life in the various races of animals is very variable and that, whereas some species are remarkably long-lived, others die after a relatively brief existence, are facts known to all. Both Weismann and Metchnikoff cite numerous instances of longevity among animals, some of the more extreme of which may be mentioned here. A sea-anemone belonging to the species *Actinia mesembryanthemum* is known to have lived for 66 years, and to have produced young, although in smaller numbers than formerly, at the age of 58. Another sea-anemone of the species *Sagartia troglodytes* lived to be 50 years old (Ashworth and Annandale, 1904). Certain marine Mollusca are said to live for as many as 100 years. Among insects there is an extraordinary variability in the duration of life, some living in a condition of maturity for only a few days or even hours, while others (certain *Hemiptera*) are believed to survive for as many as 17 years. Moreover, the duration of life is sometimes very different in the two sexes, the queen ant being known to live for several years (in one case for 15 years), whereas the male ant survives for only a few weeks.

Among fish, pike and carp are usually said to attain great ages and even to live for centuries, but there are few accurate data. A gadfish is said to have lived to be thirty (Field, 1937, 16 Oct., 985). The Reptilia have always been noted for their longevity ; crocodiles and tortoises have exceptionally long lives, a tortoise from the Galapagos Islands being stated to have lived for 175 years. A giant tortoise now at St. Helena is reported to have been there in Napoleon's time (*Observer*, 1937, 2 May).

The length of life in birds has been discussed by Gurney (1899) who cites several examples of great longevity, but the more usual duration of life is from 15 to 20 years. Canaries are stated to have attained to 20 years of age, a herring-gull to 44, an imperial eagle to 56, a heron to 60, an eagle owl to 68, a raven to 69, a swan to 70 and a goose to 80. Hobday (1920) described a pair of ring-doves which lived to be 21, and then did not die a natural death ; one which lived to be 42½ years has also been reported (*The Times*, 1934, 3 Jan.). Metchnikoff (1907) records a case of a parrot which lived for 82 years. In poultry males are much shorter-lived than females (Pease, 1947).

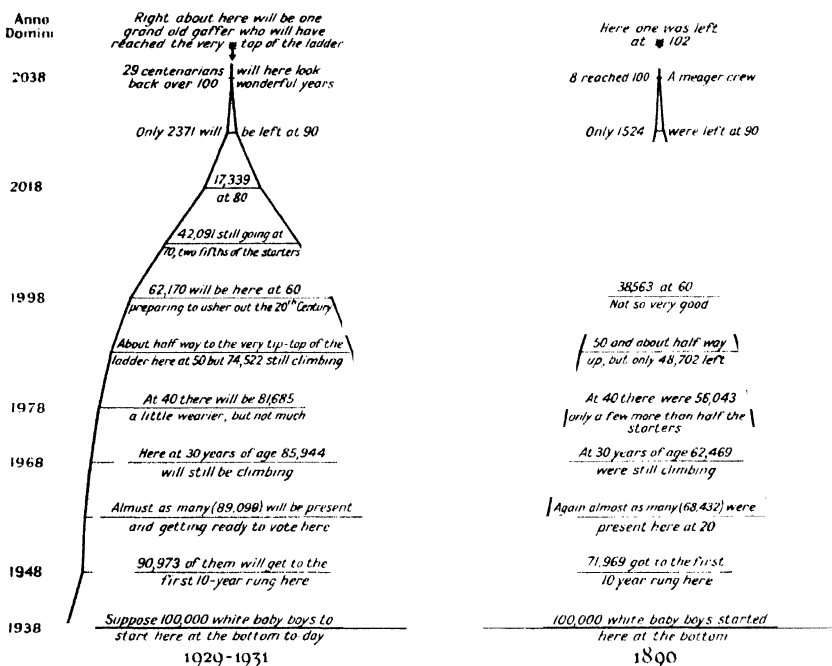
According to Weismann (1891), whales live for some hundreds of years, but it is difficult to see how this can be more than an assumption. There can be little doubt that the great age assigned by some of the older writers to elephants is mythical, and probably 150 years is almost the maximum ever attained. Horses in rare cases have reached over 40 years, cattle somewhat over 30, and sheep over 20 years. Cases of horses which lived to be 40 and 42 years old (*The Times*, 1936, 5 Aug.) and even up to 62 (*The Times*, 1930, 24 Sept., and 1932, 28 Apr.) have been reported, while a donkey which lived to be 60 has also been recorded (*Evening Standard*, 1939, 17 July). Hunter-Smith (1934) found that in a dairy herd only 5 per cent. of the cows survived up to an age of 13-16 years old, while Wright (1933) and others have shown that old age accounts for only a very small proportion of the wastage of cows from dairy herds. Cannon and Hansen (1939) found, for example, that only 0.26 per cent. of some 150,000 cows reach an age of 15 years or over. Kelley (1939), from the records of a large Merino flock, found that about 60 per cent. survived to 6 years, 30 per cent. to 10 years and 10 per cent. to 12 years old. A dog is said to have lived for 34 years, but 20 is usually regarded as a great age for this animal. A writer in the *American Kennel Gazette* (quoted from *Veterinary Medicine*, 1939) lists among dogs notable for their longevity a poodle which died at 29½ years of age; an Irish terrier which lived over 28 years; a mongrel of the Shetland sheepdog type, 27 years old; an Australian cattle dog, used as a drover's heeler in Queensland for more than 20 years, and living at the age of 26 when last heard of; and several other dogs which lived over 20 years. Cats have been known to live to be 21 and even 23, but no greater ages appear to have been recorded. The white rat may live 40 months, or even longer, but the average age appears to be about 34 months (Donaldson, 1915). According to Weisner and Sheard (1935) the mean duration of life in male rats (669 days) is shorter than in females (693 days). By retarding growth McCay *et al.* (1935) were able to increase the mean age of death in rats: members of groups retarded in growth for periods of 300-1,000 days before being allowed to grow to maturity were still alive when the last of the control groups had died at the age of 965 days (McCay *et al.*, 1939). On bringing wild rats into captivity King (1939), however, found that although maturity was attained earlier the average life span was prolonged. The white mouse is stated to have a normal life of 2 years, and this is whether it has bred or not, but females are slightly shorter lived than males (Kirkham, 1920).

In man the traditional duration of life is 70, but, as everyone knows, this age is very often much exceeded. Women on the average live to be somewhat older than men. Many instances are on record of extraordinary longevity, but perhaps the most trustworthy is the famous case of Thomas Parr,¹ described by Harvey (1700). His death is said to have been due to the change in his mode of life, resulting from his migration from Shropshire to London, "where he fed high and drunk plentifully of the best wines." "He died after he had outlived nine princes, in the 10th year of the 10th of them, at the age of 152 years and 9 months." A Bechuanaland native is said to have lived for 140 years; but this, Ernest (*The Times*, 1938, 8 Aug.) doubts, believing that the extreme life span of man to be just over 113 years—an age achieved by a French-Canadian in the nineteenth century.

¹ A portrait of Parr painted by van Dyck was to have been seen in the Royal Gallery at Dresden.

Much further information about old age, longevity and death in man may be found in the writings of Luciani (1921) and Pearl (1919, 1922).

Mammals on the average appear to have considerably shorter lives than birds. As Spencer (1899) pointed out, in birds as compared with mammals of similar size there is greater "Individuation": that this is associated with prolongation of the life span is a possibility to be considered, for in the main it is in those species which live longest that the greatest "Individuation" is found. Under modern civilisation, too, where the "Individuation" has been increased the chances of



THE LADDER OF LIFE

Fig. 23. 39—The ladder of life, as it was shaped in 1929–31 and as it was in 1890. The total span of life is much the same in each case. The width of each rung of the ladder represents the number of individuals left after each successive 10-year period after birth. Based on statistics in U.S.A. (From Pearl, 1938.)

life have also been increased. This has been brought about largely by decrease in mortality in early life, however, rather than by increasing the total span of life, which is a little over 100 years (Pearl, 1938). Pearl has shown for example, that, whereas in 1890 only 56.0 per cent. of males born lived to 40 years of age, to-day 81.6 per cent. reach that age (Fig. 23. 39): the duration of life after 40 years is, however, but slightly longer now than it was in 1890. While in ancient Rome the average life expectancy at birth was 25 years, in Rome of the 1930's it was 55 years and in U.S.A. 64 years (Pearl and Raenkham, 1932). The average expectation of life at birth in Western Europe has increased from 20 years in the sixteenth century to 25 years in the seventeenth century, 32 years in the eighteenth century, 40 years in

the 19th century, 49 years in 1900, 59 years in 1920 and nearly 62 years in 1938 (Brody, 1945). The decline in the death rate in very early life, however, is unevenly distributed, and is still high during the first two months, a fact which Waller (1939) attributes largely to defects in the mothers' milk supply.

The steps taken in recent years to control infectious diseases which were formerly the major cause of death have now shifted the major cause from those to the insidiously developing degenerative diseases (Brody, 1945). The influence of this control of infectious diseases on the age distribution of the population in U.S.A. is shown in Fig. 23. 40. Thus while in 1850 some 83 per cent. of the population were under 40 years of age, by 1980 only 52 per cent. of the population should be under this age. Similar comparisons may be made, in space, between Eastern and Western peoples. Since those under 40 are more fertile than those over 40 these facts have repercussions on the birth rate (*see* Chapter 21).

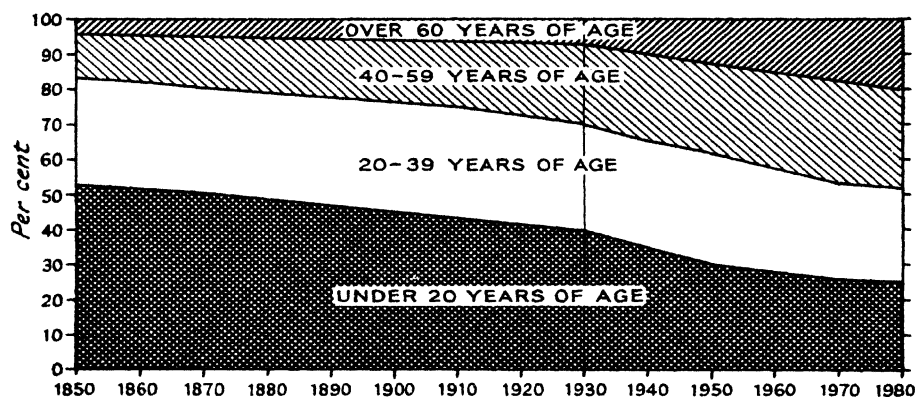


Fig. 23. 40—How control of infectious disease and death in early life has affected the age distribution of the population in U.S.A. (From Brody, 1945.)

Experimental studies on life duration, and comparison between species as well as analyses of human statistics, suggest the generalization that the length of life is generally in inverse proportion to the rate of living (Pearl, 1928, 1938). In man, Pearl's (1938) investigations point to the fact that the main consideration which determines long life is a genetic one: he agrees with Oliver Wendel Holmes, whose advice on this point was to select long-lived parents, and particularly long-lived mothers. Nonagenarians come to be such because they have organically superior constitutions, resistant to infections, and soundly organised to function efficiently as a whole organism. Pearl (1938) obtained the average physical characteristics of long-lived and short-lived groups of men and showed that the former were less of the pyknic type than the latter; in other words the long-livers tended to be more like Don Quixote and the short-livers more like Sancho Panza in their bodily structure. Of the deaths occurring in man at 90 years of age or over Pearl and Raenham (1932) find that the greater number were chargeable to breakdown of the circulatory system (45 per cent.), respiratory system (12 per cent.), kidneys (12 per cent.), and alimentary tract (7 per cent.).

As to what factors determine the average duration of life in different species is a problem about which there has been much speculation. Weismann (1891

has elaborated a theory which asserts that living matter was originally immortal, mortality first arising in correlation with cellular differentiation. On this view the Protozoa are potentially immortal, natural death occurring only among multicellular organisms. The protoplasm of the latter is considered to be of two kinds—germ-plasm, which is capable of propagating itself indefinitely under suitable conditions like the protoplasm of unicellular organisms, and somatoplasm, which composes the rest of the body and is subject to natural death. The life of the somatic cells was at first limited to one generation, but afterwards in the higher Metazoa was extended to many generations, and the life of the organism was lengthened to a corresponding degree. Such a restriction went on hand in hand with a differentiation of the parts of the organism into somatic and reproductive cells, in accordance with the principle of the physiological division of labour, and this process of differentiation was controlled by natural selection. "Death itself," says Weismann (1891), "and the longer or shorter duration of life depend entirely on adaptation. Death is not an essential attribute of living matter : it is neither necessarily associated with reproduction, nor a necessary consequence of it." According to this theory, therefore, the phenomena of senescence and death, as exhibited by all the cells of the body with the exception of the germ-cells, are secondary properties which have been preserved in multicellular organisms by natural selection, because they are of direct advantage in the propagation of the species. An indefinite prolongation of life of the organism after the age of reproduction has been passed would be of no value or utility to the race, but rather a disadvantage, since it would tend to retard the evolution of more perfectly adapted forms of life. Furthermore, according to Weismann, longevity, although depending ultimately upon the physiological properties of the cells, is capable of adaptation to the conditions of existence, and consequently is influenced by natural selection just in the same way as other specific characters are.

Perhaps the most cogent criticism of Weismann's doctrine of immortality is that of Verworn (1899), who writes as follows :

"The conception of living substances as immortal will be accepted by scarcely anyone who bears in mind the characteristic peculiarity of living substances, viz., that it continually decomposes, or, in other words, dies. There is no living substance that, so long as it is living at all, is not continually decomposing in some parts, while being regenerated in others. No living molecule is spared this decomposition. The latter, however, does not seize upon all molecules at the same time : while one is decomposing, another one is being constructed, and so on. One living particle affords the conditions for the origin of another or several others, but itself dies. The particles newly formed in turn give rise to others and, likewise, die. In this manner living substance is continually dying, without life itself becoming extinct."

From this standpoint, therefore, there can be no question of any kind of living substance being truly immortal. The whole conception of a possible immortality arises from a confusion of ideas.

Minot (1907) on the other hand, has elaborated a theory of senescence which may be regarded as an extension of that of Weismann. Like the latter, he seems to assume that death is not a universal accompaniment of life and that natural death has been acquired in the course of evolutionary development. He proceeds to define senescence as an increase in the differentiation of the protoplasm. During the early periods of life the young material is produced, and the protoplasm

is undifferentiated. During the later stages of existence cell differentiation goes on, and the organism gradually becomes old. When the cells acquire the faculty of passing beyond the simple stage to the more complete organisation they lose something of their vitality, of their power of growth, and of their possibilities of perpetuation. Just as senescence depends upon the increase and differentiation of the cytoplasm, so, conversely, rejuvenation depends upon the increase of the nuclear material; and consequently the alternation of the two phases of the life cycle (the early brief one when the young material is formed, and the later prolonged one when the process of differentiation is going on) is due to an alternation in the proportions of nucleus and protoplasm. This theory is in reality nothing more than a descriptive account of a general type of cellular change, and provides no sort of explanation as to why this change occurs, nor how it is that differentiation is apparently correlated with a reduction of vitality leading eventually to death. But it has to be remarked that work on tissue cultures outside the body (*see* Willmer, 1934) goes to show that before any differentiated tissue or type of cell can multiply and grow it has to dedifferentiate and get back to the simple form of cell. Such dedifferentiated tissue cultures, of heart and other cells, can be maintained almost indefinitely (and much beyond their normal life span in the individual) if they are periodically explanted and freed from accumulated material.

Robertson and Ray (1920) state that early growth in mice is due to over-increase of the cellular elements or parenchymatous tissue, as distinguished from the sclerous or connective tissues. They also state that early overgrowth is correlated with longer life, the individuals displaying it being highly resistant to external disturbing factors and tending towards a relative paucity of tissue accretion late in life.

Short-lived individuals, on the contrary, are relatively unstable and sensitive to external influences, and tend to display a relatively deficient early growth, but rapid and unstable accretions of connective tissue elements in late life, as in McMeekan's Low-High plane of nutrition pigs (*see* p. 820). Such accumulations are notoriously characteristic of old age, and their presence throws a strain on the metabolism of the cellular elements which support these accretions, and consequently tend to shorten life. The administration of tethelin or cholesterol, which was found to prolong life, led to an increased anabolism of the cellular elements to the disadvantage, in the competition for nutriment, of the connective tissue.

Metchnikoff (1903, 1907) laid great stress on the idea that natural death is a rare phenomenon, at least among the higher animals. That death with man is frequently, if not generally, caused by disease or accident is a fact about which there can be no disagreement and Pearson (1897) has worked out statistically the chances of death occurring in the different phases of human life. "We have five ages of man," he says, "corresponding to the periods of infancy, childhood, youth, maturity or middle age, and senility or old age. In each of these periods we see a perfectly chance distribution of mortality centering at a given age and tacking off on either side according to a perfectly clear mathematical law." It was found also that the curve of mortality, as deduced from a study of the deaths per annum of a thousand persons born in the same year "starts very high in infancy, falls to its least value at 13 or 14 years with only 2·36 deaths. It then slowly increases until it reaches a maximum in the 72nd year of life, and

falls more rapidly than it rose, till scarcely two isolated stragglers of the thousand reach 91." It is clear, therefore, that death from old age is far from being the rule in the human species, but according to Metchnikoff it seldom occurs at all.

Metchnikoff (1903) found it impossible to accept the view that the high mortality observable between the ages of 70 and 75 indicates a natural limit to human life at about this period. Centenarians, he points out, are not really very rare, and he cites many cases of extreme old age, including that of Thomas Parr referred to above. Real old age, we are told, is associated with an instinct for death which is as natural as is the instinct for sleep. Metchnikoff, therefore, answers in an emphatic negative the question asked by Admetus in Euripides' "Alkestis": "Is it the same thing for an old man as for a young man to die?" The fact that the instinct for death seems so rarely to exist is regarded as evidence that true senility is a comparatively infrequent phenomenon. According to Metchnikoff, senescence is not brought about simply as a result of arrest of the reproductive powers of the cells. The whitening of the hair in old age is due to the destructive action of phagocytes which remove the pigment. Moreover, hairs become old and white without ceasing to grow. Metchnikoff believed also that atrophy of the brain is due to the destruction of the higher nerve-cells by neurophages, and that there are many other devouring cells which are adrift in the tissues of aged men and animals and cause the destruction of other cells of the higher type. The testes, however, appear to have the power to resist these phagocytes, and with this power is correlated the fact that spermatozoa are often produced in advanced old age. Metchnikoff's theory as to the cause of death is that it is due to the poisoning of the tissues and to the damage done by phagocytes to those parts of the body affected by the toxic action. He believed further that in man and certain of the animals this process of poisoning is brought about by fermentation set up by microbial action in the large intestine. The toxic substances produced by the intestinal fermentation were supposed to enter the system and poison it, the result being that the vitality of the tissues is lowered, so that they are less able to resist the action of devouring phagocytes. The presence of lactic acid in the intestine was believed to arrest the process of fermentation. Metchnikoff recommended, therefore, the regular drinking of sour milk as a means of destroying the microbes in the intestine in the hope of prolonging life.

The term "Death," as already indicated above, is employed in two separate senses; it may mean the death of the whole body, i.e. somatic death (this being the sense in which it is ordinarily used), or it may be applied to the death of individual tissues, some of which remain alive for many hours after the body as a whole is said to be dead. The death of the body as a whole usually occurs suddenly. As Michael Foster (1891) says:

"Were the animal frame not the complicated machine we have seen it to be, death might come as a simple and gradual dissolution, the 'sans everything' being the last stage of the successive loss of fundamental powers. As it is, however, death is always more or less violent; the machine comes to an end by reason of the disorder caused by the breaking down of one of its parts. Life ceases not because the molecular powers of the whole body slacken and are lost, but because a weakness in one or other part of the machinery throws its whole working out of gear."

The synchronous disturbance of two or more of the bodily functions, such as is wont to occur in old age, may destroy that co-ordination of the various vital activities without which life cannot continue. The stoppage of the heart's beat is the ordinary criterion of death, and this is a true conception, because the cessation of the heart's movements implies the arrest of the circulation of the blood and the consequent starvation of the tissues of the body. Animals dead in this sense for short periods of time may however be brought back to life by blood perfusions with an artificial heart and lungs.

The tissues do not die simultaneously, for, as already described, some cells of the body are in process of disintegration through the whole of life. After somatic death, the cells which make up the nervous system usually die very rapidly. The same is true of the gland-cells; but the muscles may remain sensitive to external influences for many hours. This order in which tissues of the body die is probably related to their metabolic rate and so to the growth order (*see* p. 819). Kestner (1934) has, for example, shown that the brain has the highest metabolic rate of all body tissues, while the muscles have a relatively low rate of metabolism.

There is at present but little exact information as to the conditions under which the different tissues of the body may be kept alive after death of the body as a whole. It is well known that the heart, for example, after its removal from the body may continue to live and undergo rhythmical contractions for a considerable time. By keeping ovarian tissue at low temperatures and then subsequently grafting it into an animal, Lipschütz (1928) showed that it was possible to keep the tissue alive up to 16 days after death of the animal. Ram spermatozoa have by this method been kept in a motile condition for 60 days (Gunn, 1936). It has been found, however (Iwanow, 1926), that unless the sperms are removed from the body and put under low temperatures immediately after the death of the body as a whole, they die very quickly. At low temperatures naturally the metabolic rate is much reduced as compared with body temperatures and so the tissue is able to survive longer, just as during hibernation when body temperature is reduced.

In the process of death-stiffening, or *rigor mortis*, the muscles once more contract spontaneously, and not until this has happened is their life utterly extinguished. *Rigor mortis* is brought about by the coagulation of the muscle plasma within the cells. It begins at periods varying from half an hour to thirty hours after somatic death, and it continues for an average of about thirty hours. The amount of lactic acid which develops in the muscles after death depends on the amount of glycogen present and this in turn on the muscular activity which took place before death. Certain cells may even live for some time after *rigor mortis* has passed. This is notably the case with the ciliated epithelial cells of the inner surface of the respiratory passages, and with the white corpuscles of the blood. Sooner or later, however, every part of the organism perishes, putrefactive changes set in, and the entire substance of the body passes once more to that "dust" out of which its vital activities enabled it to build itself up in the progress of individual life.

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